Assessing the risks of marine debris ingestion to Procellariiform seabirds

by

Lauren Roman

Institute for Marine and Antarctic Studies

Submitted in fulfilment of the requirements for the Doctor of Philosophy in Quantitative Marine Science

University of Tasmania, November 2018

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

14/11/2018

Lauren Roman Date

Statement of Authority Access

This thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

14/11/2018

Lauren Roman Date

Abstract

Procellariiform seabirds are among the worlds most threatened species; with over half of species in population decline, and 44% of all species are threatened with extinction. There are 139 extant Procellariiform seabird species distributed globally, with the highest species density occurring in the Australasia region surrounding the Tasman Sea and Southern Ocean. Understanding the threats to seabirds is key for managing and planning for the conservation of this assemblage of threatened species.

Ingestion of anthropogenic marine debris, particularly plastics, is common among seabirds globally, and considered an emerging threat to seabird populations. Despite growing awareness of the threat of marine debris, both the incidence of debris ingestion in Australasian seabirds, and the effect of ingested plastics on seabird health and mortality is poorly understood. Though the ubiquity of marine debris ingestion in seabirds is internationally recognised, there is currently no extant baseline data on quantified health effects or mortality associated with marine debris ingestion in seabirds. The nonexistence of baseline mortality data of this potential threat poses a serious impediment to assessing risk of debris interactions, conservation planning and managing declining populations in this group of threatened seabirds.

This thesis aims to examine the factors that drive harm associated with ingestion of marine debris in Procellariiform seabirds and predict the scale of mortality expected in the current environment with current levels of marine debris pollution. Specifically, this thesis aims to examine (1) the incidence of marine debris ingestion in Australasian seabirds, (2) the mortality caused by the physical impacts of marine debris ingestion, and (3) the potential chemical and toxicological effects of ingested plastics on the growth, reproduction, and endocrine function of birds and finally (4) estimate the global seabird mortality associated with marine debris ingestion.

(1) Procellariiform seabirds were collected dead as beach-wrecked, veterinary casualties and fisheries by-catch across Australia and New Zealand between Jan 2013 and March 2017 to determine the incidence of marine debris ingestion in Australasian seabirds. Following collection, the birds were necropsied and their gastro-intestinal tracts inspected for ingested marine debris, which was removed and quantified. We collected and necropsied

1734 individual seabirds of 51 Procellariform species, covering four families of Procellariiform seabirds; *Diomedeidae*, *Procellariidae*, *Hydrobatidae* and *Pelecanoididae.* Ingestion of marine debris was found in 32% of individual birds and 31 of 51 species examined. Ingestion of marine debris was found in all Procellariiform seabird families, though we observed considerable variation in the incidence and magnitude of ingested debris among the birds examined. Some taxonomic groupings demonstrated consistent patterns in both incidence and magnitude of marine debris ingestion, while other taxonomic groupings demonstrated great variability. We demonstrated that exposure to marine debris in the environment, diet, foraging strategy and taxonomic group are all important ecological factors driving the incidence of debris ingestion in Procellariiform seabirds.

(2) Mortality resulting from physical impacts of marine debris ingestion in Procellariiform seabirds, such as gastro-intestinal obstructions and perforations, was quantified and analysed with statistical methods to establish a dose-response relationship. We found that there is a 20% chance of death from ingesting a single debris item, rising to 100% after consuming 93 items. Obstruction of the gastro-intestinal tract is the leading cause of death. Overall, balloons are the highest-risk debris item; 32 times more likely to result in death than ingesting hard plastic.

(3) The potential chemical and toxicological effects of ingested plastic on avian development, reproduction and endocrine disruption was investigated by conducting a laboratory experiment. The experiment is designed to examine the toxicological effect of ingested plastic on endocrine function in a bird species, the Japanese quail, *Coturnix japonica*. Following OCSPP 890.2100: Avian Two-generation Toxicity Test in the Japanese Quail, we designed a multi-generational plastic feeding experiment to quantify effects of plastic ingestion in a bird. Quail were experimentally fed plastic to monitor the effect on their growth, reproductive output, and endocrine function. We found that the primary adverse effect of plastic ingestion in birds is increased chance of cysts in the male reproductive tract, reduced growth in chicks fed plastic and delayed onset of sexual maturity in females, however these changes did not affect survival or reproductive output. Contrary to expectations, we did not find any other plastic ingestion effects. These results demonstrate that it’s likely that the effect of ingested plastic on a birds’ heath is primarily

driven by the physical space that it occupies and resulting dietary dilution, and that the chemical and endocrine impacts that may affect reproduction and survival are minimal.

(4) By combining the incidence of debris ingestion, mortality resulting from the physical effects of debris ingestion and experimentally determined sublethal effects from the three previous chapters and the literature, we estimated global seabird mortality from debris ingestion. We found that over 60% of examined Procellariiform species have a 5% or greater chance of dying of a debris-related cause, and 9 species having a 30% or greater chance of debris-related death. Shearwaters, fulmarine petrels and prions are the assessed groups most likely to die from debris ingestion and would benefit most from initiatives to reduce debris entering the marine environment. Our analysis confirms that plastic pollution is a serious conservation concern to threatened and declining Procellariiform seabirds.

By quantifying and modelling the estimated mortality associated with ingestion of marine debris by threatened Procellariiform seabirds, this study has provided baseline data and a framework by which to compare threats to seabirds. With this baseline data and mortality estimate, Procellariiform seabirds can be managed and conservation efforts planned accordingly in response to the marine debris pollution hazard changing through space and across time. This will be the first time internationally that the scale of the threat of marine debris to seabird populations has been quantified and can be included in analyses and decision making when planning conservation initiatives.

Statements of Publication and Co-authorship

Chapters 2,3,4 and 5 are produced from manuscripts either in final stages of preparation for, submitted to, or in review by peer-reviewed journals.

The following people and institutions contributed to the publication of the work undertaken as part of this thesis:

Lauren Roman, University of Tasmania = Candidate

Chris Wilcox, CSIRO = Author 1

Britta Denise Hardesty, CSIRO = Author 2

Mark Hindell, University of Tasmania = Author 3

Linda Lowenstine, University of California = Author 4

Laura Maeve Parsley, University of Tasmania = Author 5

Elizabeth Bell, Wildlife Management International = Author 6

Kirsten Gilardi, University of California = Author 7

Author details and their roles:

The candidate was responsible for study design, applying for and sourcing most of the funding to undertake the study, applying for required permits and licenses, sample sourcing and data collection (including making contact with partnering organisations and individuals to collect seabirds, necropsy of most of those seabirds, weighing and measuring collected marine debris, sourcing materials for the quail experiments, undertaking most of care and husbandry of the quail and collecting data through the experiment, training and supervising undergraduate volunteers assisting with the quail husbandry and data collection, performing quail necropsy, sample collection and sample and slide preparation and training and supervising undergraduate volunteers assistants), most of the data analysis, interpretation of the results, and manuscript preparation for the following manuscripts.

Paper 1 Roman, L., Bell, E., Wilcox, C., Hardesty, B. D., Hindell, M. (2018) Ecological drivers of marine debris ingestion in Procellariiform seabirds. *In review by Nature Scientific Reports.*

Chapter 2 of this thesis.

Author contributions: LR, BDH, CW, MH conceived of the study. BDH, CW and MH supervised the study. LR and EB organized, collected and necropsied the birds. LR counted the ingested plastic and prepared the data. LR, CW, MH analysed the data. LR, BDH, CW, MH and EB contributed to writing and editing of the manuscript.

Paper 2 Roman, L., Hardesty, B. D., Hindell, M., Wilcox, C. (2018) How much plastic pollution does it take to kill a seabird? *In review by Nature Scientific Reports.*

Chapter 3 of this thesis.

Author contributions: LR, BDH, CW, MH conceived of the study. BDH, CW and MH supervised the study. LR organized, collected, necropsied and determined the cause of death of the birds. LR counted and measured the ingested plastic and prepared the data. CW developed the mortality modelling methodology. LR and CW analysed the data. LR, BDH, CW, MH prepared and edited the manuscript.

Paper 3 Roman, L., Lowenstine, L., Parsley, L. M., Wilcox, C., Hardesty, B. D., Gilardi, K., Hindell, M. (2018) Is plastic ingestion in birds as toxic as we think? *Submitted to Science Advances.*

Chapter 4 of this thesis.

Author contributions: LR, BDH, CW and MH conceived of the study. BDH, CW and MH supervised the study. LR, BDH, LL, KG and MH sourced and provided resources and other in-kind support for the study. LR conducted the experiment and oversaw husbandry and cared for the quail. LR and CW oversaw the culling of the quail. LR, LL and LP prepared tissue and blood samples, performed the sample analysis and interpreted the results. LR performed the statistical analysis. LR, LL, LP, BDH, CW, KG and MH prepared and edited the manuscript.

Paper 4 Roman, L., Hardesty, B. D., Hindell, M., Wilcox, C. (2018) Estimating the global mortality of Procellariiform seabirds resulting from plastic ingestion. *In preparation for submission to Nature.*

Chapter 5 of this thesis.

Author contributions: LR, BDH, CW, MH conceived of the study. BDH, CW and MH supervised the study. LR and CW provided and prepared the data from previous works. LR and CW analysed the data, LR interpreted the results. LR, BDH, CW, MH prepared and edited the manuscript.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

Professor Mark Hindell
Supervisor
Institute for Marine and Antarctic Studies
University of Tasmania
Date:19/11/2018

Professor Craig Johnson
Head of Centre
Ecology and Biodiversity Centre, IMAS
University of Tasmania
Date 19/11/2018

Statement of ethical conduct

The research associated with this thesis abides by the Australian codes on animal experimentation, following the guidelines of the Australian Code for the Care and Use of Animals for the Scientific Purposes - 8th Edition 2013 and the rulings of the University of Tasmania's Animal Ethics Committee.

Acknowledgements

This research project comprised of two major components, one large international collection of dead seabirds, and a year-long, multigenerational laboratory animal experiment. While I'll shortly walk away with a fancy hat and some new letters beside my name, the reality is that this project was not the labour of one, but a collaboration involving hard work and hundreds of hours volunteered by many; motivated (as I am) by their love and passion for birds and wildlife. While all the people who contributed are too numerous to name, I would like to acknowledge the following organisations and individuals, whom without this project would have been less successful.

Firstly, I would like to acknowledge the numerous organisations and people across Australia and New Zealand, who over the last five years walked the beaches, picked up dead birds, filled their freezers or allowing me to come and stink up their space with carcasses. I would like to thank the Auckland War Memorial Museum, Australia Zoo Wildlife Hospital, Australian Museum, Australian Seabird Rescue Inc, BirdLife Australia, BirdLife Tasmania, Bonorong Wildlife Sanctuary, Currumbin Sanctuary Wildlife Hospital, Department of Conservation (New Zealand), Department of Environment and Heritage Protection (QLD Australia), Department of National Parks, Recreation, Sport and Racing (QLD Australia), Department of Primary Industries, Parks, Water and Environment (TAS Australia), Department of Parks and Wildlife (WA Australia), Friends of Shorebirds SA, Holistic Vets Tauranga, Melbourne Museum, Moreton Bay Research Station, the Ornithological Society of New Zealand, Pelican and Seabird Rescue, Queensland Museum, RSPCA QLD Wildlife Hospital, South Australian Museum, Tasmanian Museum and Art Gallery, Te Papa Museum, University of Queensland, University of Western Australia and Wildlife Management International. I would also like to thank the following people who have assisted in bird collection: Belinda Bauer, Linda Behrendorff, Elizabeth Bell, Bentley Bird, Tanya Bishop, Stephanie Borelli, Bianca Burford, Kris Carlyon, Mike Clarkson, Peter Collins, Bonny Cumming, Lloyd Esler, Rochelle Ferris, Lesley Feasey, Kim Fleischfresser, Hammy Forrest, Nat Forrest, Alan Gennings, Amber Gillet, Isabella Godbert, Petra Harris, Grace Heathcote, Mike Hines, Philippa Horton, Greg Irons, Heather Janetzki, Claude Lacasse, Julia Lesel, Kevin Matthews, C. Mischela, Harriet Patterson, Liz Pulo, Mic Pyne, Matt Rayner, Philip Rhodes, Heather Rogers, Stephanie Shaw, Jaynia Sladek, Luke Short, Ian Southey, Kathrina

Southwell, David Stewart, Liz Schneider, Patricia Swift, Paige Taylor, Alan Tennyson, Kathy Townsend, Leah Tsang and Eric Woehler.

Secondly, the husbandry and daily care of hundreds of quail over a year was an enormous undertaking, and one that I did not do alone. I am eternally grateful to UTas Zoology and Antarctic and Marine Science undergraduate students: Gabby Walley, Alexandra Johne, Harmon Overall, Matthew Rose, Juliana Chan, Megan Husband, Samantha Seah, Misaki Kawamura, Yieh Shing Tan, Deirdre Merry and Damien Ashlin, and Dr. Catherine Young. Thank you for your hours of help feeding quails, cleaning bowls, weighing quails, collecting eggs, breaking eggs, scooping poop, sweeping floors, in the sad yet hectic cull days, performing necropsies, collecting and spinning blood, and preparing histology slides. This experiment would not have been possible without your enormous contribution across the course of this experiment. Further to this, I would like to acknowledge the 249 Japanese quail who made the ultimate sacrifice of their lives in my pursuit of understanding the effects that plastic is having on the millions of wild birds that ingest it. Unlike the rest of us, you didn't sign up for this; there is no sugar-coating this fact and I acknowledge responsibility. I hope that your short life wasn't too unpleasant, and that you are frolicking in endless dust-baths in quail heaven.

I would like to acknowledge UTas veterinarian Dr. Jane Dunnett, who was always so responsive and helpful even when I inundated you with messages about sick quails at unsociable hours, and Dr. James Harris, for your training and willingness to help wherever you could. I would like to thank the UTas Animal Ethics committee, particularly Mel Perry (and probably others in the background whose names I'll never know), who said 'yes' to spending considerable time in a slow and clunky system, putting new procedures in place for rehoming of surplus animals, when it would have been much easier to say 'no'.

Thank you to the lab managers Toby Bolton, Anna McEldowney and Lisette Robertson, who despite some of the curly projects and requests I approached you with across both projects, always found a way to make the sometimes impossible happen. You were asked a million questions, had your lab spaces taken over and filled with dreadful smells, but you were nothing but supportive and helpful and were a pleasure to work with throughout the project.

Thank you to the Australian Government's Australian Postgraduate Awards scheme, which kept me fed and housed the past three and a half years. Thank you to my work boss Greg Milne at Hobart City Council, who provided the most flexible working arrangement I could possibly ask for during the last three years while undertaking this study, contributing to my feeding and housing. Thank you to the Holsworth Wildlife Research Endowment, the Australian Wildlife Society, BirdLife Australia, BirdLife Tasmania, Sea World Research and Rescue Foundation, CSIRO and the University of Tasmania, who financially supported the project costs that allowed this study to take place.

Thank you to my supervisors, Dr. Denise Hardesty, Dr. Chris Wilcox and Prof. Mark Hindell, for your expertise, support and guidance throughout this entire project, as well as your invaluable advice in manuscript revisions and edits. Thank you also to my co-authors Dr. Elizabeth Bell, Prof. Linda Lowenstine, Dr. Laura Parsley and Dr. Kirsten Gilardi for your contributions to this project. Thank you to my BSc honours supervisor Dr. Kathy Townsend, who indulged my random request six years ago to investigate marine debris in Australian birds, taught me to necropsy a bird, and started me on the path that lead to this thesis.

Thank you to my parents for the early opportunities that provided a foundation to pursue my interest in science and wildlife.

And finally, thank you to my partner Sean for your ongoing support, acceptance of detours to look for dead birds, taking care of everything at home during my sometimes-long absences, and building more bird housings than you probably thought conceivable in a lifetime.

Table of Contents

Contents

Declaration of Originality 2
Statement of Authority Access 2
Abstract 3
Statements of Publication and Co-authorship 6
Author details and their roles: 6
- Paper 1 7
- Paper 2 7
- Paper 3 7
- Paper 4 8

Statement of ethical conduct 8
Acknowledgements 9
Table of Contents 12
- Contents 12

Chapter 1: General introduction 14
- Marine debris is a global issue 14
- Marine debris ingestion in seabirds 14
- The Procellariiform seabirds 15
- Effect of marine debris ingestion on avian health 15
- Known impacts- gastrointestinal obstruction and perforation caused by marine debris 16
- Dietary dilution and its effect on body condition 16
- Potential health effects of endocrine disrupting chemicals and organic contaminants of marine plastics 17
- Organic contaminants of marine plastics 18
- The effect of plastic ingestion on seabird populations 19
- Aims 19

Chapter 2: Ecological drivers of marine debris ingestion in Procellariiform Seabirds 22
- Abstract 22
- Introduction 23
- Methods 24
- Results 26
- Discussion 28
- Figures 33
- Supplementary information 38

Chapter 3: How much plastic pollution does it take to kill a seabird? 43
Abstract 43
Introduction 43
Methods 45
Results 47
Discussion 49
Figures 51
Chapter 4: Is plastic ingestion in birds as toxic as we think? 57
Abstract 57
Introduction 58
Methods 58
Hormone analysis 61
Statistical analysis 61
Results 62
Discussion 63
Figures 65
Supplementary Materials 69
Discussion Chapter: Estimating the global mortality of Procellariiform seabirds resulting from plastic ingestion 74
Summary 74
General discussion 75
Figures 78
Supplementary information 85
References 87

Chapter 1: General introduction

Marine debris is a global issue

One of the most widespread and pervasive transformations of the global marine environment in recent history is pollution by anthropogenic marine debris [1, 3]. The majority of marine debris, defined as discarded solid synthetic polymers, is dispersed from population centres on oceanic currents, and is consequently abundant even in remote areas uninhabited by humans [4, 5] where it can remain for hundreds of years [3]. Buoyant marine debris occurs at or near the surface of the ocean, where it is vertically mixed by oceanic currents and wind [6]. Plastics comprise 60-80% of debris in the world's oceans, and in excess of 90% in some regions [7]. An estimated 5 trillion pieces of plastic together weighing 250,000 tonnes is estimated to be in the ocean [8]. The most common type of marine debris at the ocean's surface are polypropylene and polyethylene plastic fragments less than 10mm in size, and weighing less than 0.05g [9]. Wildlife foraging at and near the surface of the ocean encounter and ingest floating marine debris in this microplastic size range.

Marine debris ingestion in seabirds

Ingestion of anthropogenic debris from the marine environment is widespread among seabirds [7, 10], which mistake the floating plastics and other debris for food [11]. Half of all seabird species ingest marine debris, a figure expected to rise to 99% of all species by 2050 [1], yet we know little of the effect that these plastic loads have on the tens to hundreds of millions of seabirds carrying them.

The incidence (defined as percentage of affected individuals in a group), type and retention of marine debris varies among seabird species [12]. In Australasia, Procellariiformes is the order in which species most commonly ingests debris, with 65% of species having ingested marine debris [12], greater than double other examined Australian marine bird orders (Suliformes 25%, Charadriiformes 21.4%, Pelecaniformes 11.1%)[12]. Rates of debris ingestion also vary within the Procellarriiform order, with plastic ingestion rates ranging from near 0% in *Diomedea* and *Thalassarche* albatrosses [10], to in excess of 90% reported among populations of species including Cory's shearwater, *Calonectris diomede*a [13], short-tailed shearwater, *Ardenna tenuirostris* [12] and Northern fulmar, *Fulmarus glacialis* [14].

The Procellariiform seabirds

The Procellariiformes contains the albatrosses, petrels and shearwaters and are often collectively referred to as the 'tubenosed' seabirds for the often conspicuous external structure surrounding their nares [15]. Procellariiform seabirds are a higher-order predator in the marine environment, feeding primarily on fish, cephalopods and crustaceans [16]. They are a long-lived and predominantly oceanic group of species , coming to land only to breed [16]. The order contains some of the world's most abundant bird species, including the Wilson's storm petrel, *Oceanites oceanicus*, and the short-tailed shearwater, *Ardenna tenuirostris* [15, 16].

There are 139 species of Procellariform seabird globally, with the highest species diversity (n=90) occurring in the Australasia region [17]. Of these 37 are threatened (listed as vulnerable, endangered, or critically endangered), and 14 are near threatened [17]. There are few baseline data regarding which species are affected by marine debris ingestion, with only 20 of these 90 species having been systematically studied for marine debris ingestion in this geographic region [12]. Among the species examined, debris ingestion has been recorded in 65% of species, although the true percentage is likely to be higher as some of these species are represented by single negative records.

Procellariiform seabirds are considered to be at higher risk of ingesting and retaining marine debris than other avian orders due to a combination of their life history, ecology and morphology [12]. Procellariiform birds forage visually [18], concentrating their search effort within 6m of the surface of the ocean, which is also where buoyant marine debris occurs [16, 19]. Items such as hard plastic fragments, pellets, and latex balloons, resemble the squid and crustaceans seabirds feed upon[12], and these are selectively ingested [12]. The morphology of their gut, with an isthmus juncture located between the proventriculus and the gizzard prevents the regurgitation of some ingested items once they have entered the gizzard [20].

Effect of marine debris ingestion on avian health

Little data on the effect of debris ingestion on avian health exist. Most of the potential health effects of marine debris ingestion originate from a combination of speculation based on ingestion rates or associated chemical effects studied in non-avian species, combined with handful of wild bird necropsy and mortality records [7, 21-23]. Where data exist, they are often incidental records or observations of damage to birds found dead and having contained ingested plastic on necropsy. There have been two experiments examining the effects of plastic ingestion. In the first experiment, Ryan and Jackson (1987) experimentally fed polyethylene pellets to white-chinned petrel, *Procellaria aequinoctialis*, chicks and found no difference between those fed plastic and the control group [24]. In the second experiment, Ryan (1988) experimentally fed polyethylene pellets to 14 day old domestic chickens, *Gallus domesticus*, reporting impaired feeding activity and slowed growth [25].

Known impacts- gastrointestinal obstruction and perforation caused by marine debris

Published records of health effects of ingested debris in birds are limited but include a number of recorded physical health effects. Perforation of the gizzard wall by hard plastics has been recorded in two fledgling short-tailed shearwaters [26]. Reduced fat deposition has been recorded in red phalaropes, *Phalaropus fulicarius*, containing ingested plastic [22]. Localised proventricular ulceration surrounding large ingested plastic items has been recorded in three great shearwaters, *Ardenna gravis*, a cape petrel, *Daption capense*, and blue petrel, *Halobaena caerulea*; and obstruction and subsequent starvation caused by marine debris ingestion has been observed in a Northern gannet, *Morrus bassanus,* and great shearwater [21]. Though records of physical effects of debris ingestion in birds are scarce, there is evidence from sea turtles that suggest that physical effects, though likely infrequent, may be of conservation concern via gut impaction and necrosis [27] and dietary dilution [28].

Data concerning avian marine debris ingestion is often collected opportunistically. Carcasses can be in varying stages of decomposition and there may be multiple pathologies, making identification of the cause of death difficult. As a result, health effects and deaths from marine debris ingestion are likely under-reported. Thus, it is difficult to accurately quantify the scale of mortality resulting from marine debris ingestion. The small number of mortality records, even taking under-reporting into consideration, suggest that such deaths are probably infrequent.

Dietary dilution and its effect on body condition

Ryan (1988) experimentally demonstrated dietary dilution from polyethylene pellet ingestion in domestic chicks, *Gallus domesticus*, presumably due to decreased gizzard volume available for food storage [25]. Such decreased growth leads to lower fledging weight and later timing of fledging in wild birds [29, 30]. This suggests that dietary dilution from plastic ingestion, which leads to reduced growth in chicks, may have direct survival implications in wild birds, as smaller and later-fledging chicks have been observed to experience higher mortality than those with normal fledging weights and timings [29, 30].

Although dietary dilution is a possible mechanism leading to reduced growth, and reduced fitness in affected birds, this has never been directly examined. Therefore, it is difficult to draw conclusions about the effect of ingested plastics on condition of wild birds, as there are many confounding factors associated with life history that also affect body condition. In a small sample of red phalarope, body condition was negatively correlated with load of ingested plastic [22]. However, in adult short-tailed shearwaters, the presence of plastic was not correlated to body condition [31]. These contradicting observations demonstrate the need for controlled or experimental examination of the effect of ingested plastic on avian body condition.

Potential health effects of endocrine disrupting chemicals and organic contaminants of marine plastics

One of the concerns for animals ingesting plastics is exposure to the chemicals associated with the plastics. There are two major concerns; (i) exposure to endocrine disrupting chemicals, such as phthalates and polybrominated diphenyl esters (PDBEs) that are used in the manufacture of plastics, and (ii) the transfer of organic contaminants sorbed in the marine environment from the surface of the plastic to the body and tissues of the animal.

Phthalate ester plasticizers and PDBE flame retardants, are two groups of chemicals used in the manufacture of plastics which are also common pollutants in the marine environment [32, 33]. Phthalates are not chemically bound to plastic polymer matrices and readily leach into the environment [34]. Phthalates act as endocrine disruptors and interfere with the function of hormone systems and other biological pathways, even at low concentrations [35].

Endocrine disrupting chemicals in mammals can cause negative developmental, neurological, cardiovascular, reproductive, metabolic and immune effects, and increase susceptibility to some diseases [36]. In female mice, exposure to phthalates affects both pregnancy and offspring; it decreases the incidence of pregnancy, reduces implantations, increases resorptions and decreases the foetal weights [37, 38].

Much of the research on the effects of endocrine disrupting chemicals has been on mammals, fish and invertebrates [35, 36], with little on birds. Male Japanese quails exposed to di(n-butyl) phthalate (DBP) produced poorly developed or mis-shaped testes, and altered spermatogenesis resulting from tubular degeneration and atrophy of seminiferous tubules [39]. PDBEs occur in the abdominal adipose of short-tailed shearwaters which have ingested plastic, suggesting the transfer from the plastic to body tissue [40]. PBDEs have been demonstrated in the laboratory to transfer from plastics into seabird stomach oil by leaching [41]. Despite this potential pathway the resulting health effects have yet to be explored.

PDBE exposure has been observed to result in multiple physiological and behavioural aberrations in American kestrels (*Falco sparverius*) [42, 43]. Male American kestrels exposed to PDBEs in-ovo had poorer reproductive success at maturity; including less copulation attempts, fewer mate calls, failure to lay, smaller clutch sizes and smaller eggs [42]. PDBE exposed embryonic and nestling American Kestrels were associated with changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress [43] and changes is plasma retinol in adults [44].

We suggest that the scarcity of evidence of health consequences in plastic exposed birds may result from a lack of targeted studies rather than a lack of negative outcomes of exposure, which demonstrates the importance of experimental research investigating health effects in birds that ingest plastic.

Organic contaminants of marine plastics

In the marine environment, plastics sorb persistent, bio-accumulative, and toxic substances (PBTs) from seawater [45, 46]. PBTs such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dichloro-diphenyl-trichloroethane and its metabolites (DDTs), polybrominated diphenyl ethers (PBDEs), alkylphenols and bisphenol A have been detected in fragments of marine plastics at concentrations ranging from 1 to 10,000 ng/g [46]. These PBTs may desorb when the plastic is ingested by marine animals. While there is concern about plastic as a vector for PBTs, recent modelling has shown that environmental intake from food and water is the main source of exposure for some PBTs including Phe, DEHP and DDT, and input from ingested plastic was negligible [47].

The effect of PBT contamination from plastics has been demonstrated experimentally in several species. Liver toxicity and pathology has been demonstrated experimentally in a fish, Japanese medaka (*Oryzias latipes*) [48]. Among invertebrates, transfer of 19 PCBs to the marine lugworm, *Arenicola marina*, experimentally fed microplastic polystyrene caused statistically significant reductions in feeding, weight and fitness [49]. In seabirds, the mass of plastic ingested by short-tailed shearwaters correlated with the concentration of lower chlorinated PCB congeners in abdominal adipose tissue[31].

The effect of plastic ingestion on seabird populations

Despite the potential risks that marine debris poses to Procellariiformes globally, there are major gaps in our knowledge of its effects on seabirds. In 2014, an expert panel listed priority marine debris research areas, including species-level and population-level impacts of plastic pollution and whether they can be quantified [50]. While some of the threats that ingested plastics pose to avifauna are well known, no one has yet determined how serious these risks are to individual health, mortality, and the consequent effects on seabird populations. As a result, the degree to which ingested plastics are a problem that requires conservation planning and management is unknown.

Aims

This thesis aims to answer several key questions as a pre-requisite to assessing the risk of marine debris ingestion to Australasian Procellariiform populations: (i) what are the ecological drivers of marine debris ingestion in Australasian seabirds, (ii) how much ingested plastic does it take to kill a seabird, (iii) how toxic is plastic ingestion in birds and what are the sublethal effects, and (iv) what is the estimated seabird mortality from debris ingestion?

These objectives were achieved by: an assessment of the frequency of marine debris ingestion in wild Australasian seabirds; an assessment of the frequency of marine debris ingestion as a direct cause of mortality in seabirds; a laboratory experiment to investigate potential chemical effects of plastic ingestion; and a mortality estimate that combines these original research findings with global marine debris and seabird ingestion incidence from the literature. This thesis is organised into four research chapters, each addressing a key research question required to assess the risk of marine debris ingestion to seabirds. Each chapter is outlined below.

<u>Chapter 2</u>- Ecological drivers of marine debris ingestion in Procellariiform seabirds
Aim: Quantify the incidence of debris ingestion in Australasian Procellariiform seabirds and determine which aspects of a seabirds' ecology influence the incidence of marine debris ingestion in an individual seabird. Dead seabirds were collected from around Australia and New Zealand and necropsied to determine whether the bird had ingested marine debris and if so, how much was ingested. This information provided a baseline of current incidence of debris ingestion in Australasian seabirds. Potential factors investigated included: taxonomic group, diet, foraging behaviour and foraging location. The percentage incidence and number of debris items were compared to determine which aspects of a seabirds' life history influence their incidence of marine debris ingestion.

<u>Chapter 3</u>- How much plastic pollution does it take to kill a seabird?
Aim: Determine how much plastic it takes to kill a seabird by developing a dose-response relationship between debris load ingestion and seabird death. The cause of death of all seabirds necropsied in Chapter 1, along with load of debris ingested by each individual bird. We used the amount of ingested debris as a response variable, and compared the marine debris loads in birds whose cause of death was i) unrelated to plastic (*e.g.,* death due to fisheries by-catch or injury), ii) those with unknown causes of death and iii) resulted from ingested marine debris (*e.g*., gastro-intestinal tract blockage). Using this information, we used statistical methods to determine the dose-response relationship between ingested marine debris load and chance of death.

<u>Chapter 4</u>- Is plastic ingestion in birds as toxic as we think?
Aim: Quantify the effects of plastic associated chemicals on a bird's health and to determine whether plastic ingestion affects development, reproduction, and endocrine disruption in a model bird species. Biologically relevant loads of plastic were fed to Japanese quail, and the impacts on development, reproduction, and endocrine disruption were recorded over three

generations. Answering these key questions and quantifying mortality and sub-lethal effects will provide information to model mortality resulting from the ingestion of marine debris by seabirds.

<u>Discussion chapter:</u> *Estimating the global mortality of Procellariiform seabirds resulting from plastic ingestion*

Aim: Combine the findings about ecological drivers of plastic ingestion (Chapter 2), mortality rates from the physical effects of plastic ingestion (Chapter 3), and sub-lethal effects (Chapter 4); with global marine debris density data from the literature, to estimate the global seabird mortality from plastic pollution.

Chapter 2: Ecological drivers of marine debris ingestion in Procellariiform Seabirds

Lauren Roman[1], Elizabeth Bell[2], Chris Wilcox[3], Britta Denise Hardesty[3], Mark Hindell[1, 4]

1 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia,

2 Wildlife Management International, Blenheim, New Zealand,

3 CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia,

4. Antarctic Climate and Ecosystems CRC, University of Tasmania, Hobart, Tasmania, Australia,

Abstract

Procellariiform seabirds are both the most threatened bird group globally, and the group with the highest incidence of marine debris ingestion. We examined the incidence of and ecological factors associated with marine debris ingestion in Procellariiformes by examining seabirds collected at a global seabird hotspot, the Australasian - Southern Ocean boundary. We examined marine debris ingestion trends in 1734 individuals of 51 Procellariform species, finding significant variation in the incidence of marine debris abundance among species. Variation in the incidence of marine debris ingestion between species was influenced by the taxonomy, foraging ecology, diet, and foraging range overlaps with oceanic regions polluted with marine debris. Among the ecological drivers of variability in marine debris ingestion in Procellariiformes, we demonstrated that the combination of taxonomy, foraging method, diet, and exposure to marine debris are the most important determinants of incidence of ingestion. We use these results to develop a global forecast of taxa at the risk of highest incidence of marine debris ingestion. We found that seabirds with the highest risk of debris ingestion foraged at the surface, especially by surface seizing, diving and filtering, had a crustacean dominant diet, and foraged in or near marine debris

hotspots. The family with the highest risk is the storm petrels (*Hydrobatidae*). We demonstrate that the greater the exposure of high-risk groups to marine debris while foraging, the greater the incidence and number of marine debris items will be ingested.

Introduction

Ingestion of plastics and other marine debris in the marine environment is a widespread, emerging threat to seabirds, [7, 10] which mistake plastics for food [11]. Presently, 50% of the world's seabird species have been reported to be affected by marine debris ingestion [51]. With an estimated 5 trillion plastic pieces currently floating in the world's oceans [8], and more entering daily, floating plastics and other marine debris pose a growing risk to seabirds [7].

Seabirds predicted to be at greatest risk are those within the Southern Ocean boundary, particularly surrounding the Tasman sea between Australia and New Zealand, as it is an identified hotspot for risk of debris ingestion in seabirds [1]. Multi-species studies investigating plastic consumption in aquatic and marine birds report that Procellariiformes ingest marine debris at greater frequency than the eight other avifauna orders studied [12, 52]. There are 90 species of Procellariiform seabirds in the Oceania region surrounding the Southern Ocean and Tasman sea boundary, of which 37 species are threatened (listed as vulnerable, endangered, or critically endangered on the IUCN red list [17]), 14 are near threatened. Of these species, over half (54%) are in population decline [17].

The rates of plastic ingestion vary substantially between species. *Diomedea* and *Thalassarche* albatrosses rarely ingest plastics [53], while plastic ingestion rates can exceed 90% in other species, including Cory's shearwater, *Calonectris diomed*ea [13], Northern fulmar*, Fulmarus glacialis* [14], and short-tailed shearwater, *Ardenna tenuirostris* [12]. Data explicitly examining plastic ingestion in seabirds globally is patchy, with comprehensive data available for just a small proportion of common or accessible species. Species exposure risk modelling attempts to fill these knowledge gaps, with recent modelling suggesting that the incidence of debris ingestion in seabirds increases with increasing exposure, foraging strategy and recent timing of study [1].

Variation in the incidence of marine debris ingestion has been attributed to species' geography [53] and foraging behaviour [54], with more ingestion in species with zooplankton diets and surface feeding [12]. However, there has yet to be a synoptic, comprehensive, multi-species study of Procellariiformes examining the relative contribution of potential drivers which may put some species at greater risk of marine debris ingestion. In this study, we evaluated the relative importance of ecological drivers of debris ingestion in Procellariiform seabirds and used this information to predict which seabird groups have the highest risk of debris ingestion. Using the incidence of debris ingestion across 51 Procellariiform species, we determined a set of ecological criteria useful for predicting risk of marine debris ingestion.

Methods

Whole dead seabirds were obtained from fisheries by-catch, veterinary casualties and beach-washed carcasses from Australia and New Zealand between February 2013 and February 2017. Collections of deceased birds spanned from Perth, Western Australia in the West to Chatham Rise, off New Zealand in the east and from Fraser Is in the north to Macquarie in the south (Figure 1). All methods were carried out in accordance with relevant guidelines and regulations

The birds were necropsied according to well-established collection and dissection procedures [55]. The age was recorded, and contents of the proventriculus and gizzard were removed and carefully visually inspected for anthropogenic debris (items of a natural origin such as pumice, squid beak, fish bone, shell, insect, seed and wood were excluded from analysis). Debris which was visible to the naked eye was removed, rinsed in distilled water, and air dried before being stored in aluminium foil for manual counting and further analysis.

Each seabird species was identified to species level and then categorized by genus, foraging range overlap with marine debris hotspots, presence or absence of constricted juncture between the proventriculus and gizzard (absent in albatross and present in other Procellariiformes), primary diet and primary foraging behaviours. Diet categories included cephalopods, fish, crustaceans or scavenging. Foraging categories were surface diving, surface seizing, surface plunging, surface filtering, pursuit diving, pursuit plunging, pattering, hydroplaning, and dipping. Each diet and foraging method was recorded as "major importance", "minor importance", and "absent or rare", for each species following

Marchant and Higgins 1990 [16]. We used the R (Version 3.3.3) [56] package "Rmixmod" cluster analysis to group species into diet clusters and foraging clusters, choosing the best number of clusters based on maximizing the log likelihood of the model. Marine debris hotspots followed Wilcox et al. 2015 [1], and a diet cluster and foraging cluster was assigned to each species. Where range, diet and/or foraging behaviour were unknown, these species were excluded from associated analysis (SI Table 1).

Seabird species distribution and expected habitat use data were sourced from BirdLife International's seabird database [2] and the debris density was determined following Wilcox et al. 2015 [1]. In brief, the 'encounter density' rate at which a seabird was likely to encounter debris within its foraging range was determined by multiplying the seabirds' expected habitat use with the debris density in that location. Each species was allocated an 'encounter density' value, which was the sum of the debris density multiplied by the species' expected use (the weighted distance from the edge of the range) of each 1° latitude by 1° longitude grid across its foraging range. Encounter density represents how many debris items a species would be expected to encounter during its average foraging activities.

Statistical analyses were performed using R (Version 3.3.3) [56]. We used generalized additive models (GAMs) to determine whether the sum of ingested debris items was significantly influenced by any of the factors described above. To determine the best model to explain the variation in the number of marine debris items ingested by a seabird, we used the "dredge" function in the "MuMIn" package to compare all possible factors, excluding species to prevent overfitting, and chose the model with the lowest Akaike information criterion (AIC). With this best model we tested interactions between ecological factors to obtain the best model to use for predicting marine debris ingestion in seabirds.

To support our analysis, we used three species case studies of species with a known geographical foraging range. The first case study examines two species, fluttering shearwater and fairy prion, which inhabit an overlapping range in the Tasman sea and share a similar diet but dissimilar foraging method to demonstrate the influence of foraging method. The second case study examines the ranges of two geographically separated populations of flesh-footed shearwaters, *Ardenna carneipes*, supported by tracking data [57, 58], are used as a case study of drivers of variation in debris ingestion within a species. A third case study examines a speciesthat follows a known migration route, short-tailed shearwater, and provides evidence of drivers of variation in debris ingestion across geography within a taxonomic group.

Results

Incidence and magnitude of marine debris ingestion

We collected and necropsied 1734 individual adult and immature seabirds of 51 species, representing all four families of Procellariiform seabirds; *Diomedeidae*, *Procellariidae*, *Hydrobatidae* and *Pelecanoididae*. All four families studied ingested debris (SI Table 1). Overall, debris was recorded in 32% of individuals and 31 of 51 species examined, with the highest number of items ingested by an individual bird being 40 items. Among individuals that had ingested debris, a mean of 4.95 and a median of 3 items were ingested (SI Table 1).

Ecological drivers of marine debris ingestion in Procellariiformes

When examined in isolation, species is the best single predictor of number of ingested marine debris items, with 50.1% of deviance explained (R^2=0.281).

A cluster analysis of foraging behaviour grouped the species into seven foraging clusters (Log-likelihood = -4571.6). Foraging groups can be broadly described as: mostly surface diving and pursuit diving/plunging (group 1), mixed surface foraging including filtering, seizing, plunging and dipping (group 2), feeding under the surface by pursuit diving/plunging (group 3), surface seizing and diving (group 4), mostly pursuit plunging with some surface and pursuit diving (group 5), surface seizing and plunging with minimal other feeding methods (group 6) and surface seizing, pattering and dipping (group 7). Foraging method explained 32.6% of the deviance (R^2=0.187) in the number of items and was the next single best predictor. Foraging groups 1, 2, 3, 4 and 7 had significant variation in the number of ingested debris items. While all foraging groups ingested marine debris, groups 1 and 2 had greater ingestion compared to other foraging groups (Figure 2).

Taxonomic grouping significantly influenced the number of items ingested by a seabird for albatrosses, fulmarine, gadfly and giant petrels, shearwaters, prions and storm petrels, explaining 31.7% of the deviance in number of items ingested (R^2=0.129) (Figure 3). Storm-petrels ingested the most marine debris (median =13, standard deviation =8.1), followed by fulmarine petrels (median=2, standard deviation =4.6), and giant petrels (median =1, standard deviation = 7.5). Procellarine petrels (median=0, standard deviation =0.2) ingested the least marine debris, followed by diving petrels (median=0, standard deviation =0.4) (Figure 3).

Cluster analysis sorted birds' diets into six diet clusters (Log-likelihood = -3641.9). Diet groupings can be generally described as: squid dominant, with some fish, crustaceans and scavenging (group 1), fish dominant, but some squid and crustaceans (group 2), crustacean dominant but may take fish and squid (group 3), cephalopods and crustaceans dominant, with some also taking fish (group 4), mostly fish and crustaceans (group 5), and mostly fish and squid with some scavenging (group 6). Diet grouping explained 30.8% of the deviance in debris ingested (R^2=0.154), and diet groups 1, 3, 4, 5 and 6 were significantly correlated with marine debris ingestion. While all diet groups ingested marine debris, diet groups 3 (median =2, standard deviation = 3.67) and 4 (median =2, standard deviation = 5.13) ingested the highest number of items (Figure 4).

Encounter density was significantly positively correlated with the number of debris items ingested by a seabird, explaining 11.4% of deviance in ingested items (R^2=0.154). The deviance explained by encounter density did not overlap with the deviance explained by diet and foraging method.

<u>The best model to predict marine debris ingestion in Procellariiform seabirds</u>

The best model to explain the number of debris items ingested by a seabird includes taxonomic group, diet group, foraging group and encounter density (df 22, log likelihood -4199.65, AIC 8443.9), explaining 48.9% of deviance. The model including the presence/absence of an isthmus juncture in the gastrointestinal tract is equivalent to this best model (df 22, log likelihood -4199.65, AIC 8443.9).

<u>Case studies</u>

1) Fluttering shearwater and fairy prions inhabit a large overlapping range in the Tasman sea [15]. Both fluttering shearwater and fairy prion were allocated diet group 5 (diet mostly fish and crustaceans) by dietary cluster analysis. Fluttering shearwater was allocated foraging group 3 (feeding under the surface by pursuit

diving/plunging) and fairy prion was allocated foraging group 7 (surface seizing, pattering and dipping) by foraging strategy cluster analysis. An ANOVA demonstrates that fluttering shearwater (n=70, mean debris ingested = 0.16±0.5 items) ingest significantly less (P=0.02) marine debris than fairy prions (n=236, mean debris ingested = 0.63±1.6 items).

2) Flesh-footed shearwater foraging range was split at longitude 145 into an eastern and western foraging population. The eastern population was significantly (AIC=223.83, P<0.01) more likely to ingest marine debris, with 59.4% debris ingestion, than the western population with 18.75% debris ingestion. There is a significant difference (R^2=0.158, P<0.01) in the number of items ingested by flesh-footed shearwaters from eastern (median=2, standard deviation=6.94) and western populations (median=0, standard deviation=1.06) (Figure 5). The ratio of expected use of foraging habitat, as per Wilcox (2015) east of longitude 145 to west of longitude 145 is 0.57:1. After accounting for expected use, the adjusted encounter density ratio for the eastern and western population is 1:0.501.
3) A subset of short-tailed shearwaters (n=201) were collected during a 2013 November and December wreck during their return migration. Birds sampled from this wreck had ingested an average of 4.43 (±4.17) items.

Discussion

Debris ingestion by an individual seabird can be predicted by its foraging strategy, taxonomic grouping, diet and the environmental exposure to marine debris pollution, demonstrating that the ecological drivers of marine debris ingestion are complex and cannot be attributed to a single variable. Understanding the relative contributions of these ecological factors to the incidence of marine debris ingestion is useful for forecasting seabird species at high risk of marine debris ingestion – and the geographic areas of highest risk. Taxonomy (*e.g.* species) is the best single predictor variable of marine debris ingestion, with species capturing over half of all variation in debris ingestion in this study, suggesting that incidence of debris ingestion within a species can be predicted if incidence is known in a subset of that species.

Though debris ingestion occurred across all foraging groups, those that forage below the sea surface and by pursuit of prey (groups 3 and 4) were at the lowest risk of ingesting marine debris. This may be due to buoyant marine debris occurring mostly at the ocean's surface, and passively floating debris may not trigger the instinct of a bird which forages by pursuit. Mixed surface feeding strategies were associated with higher levels of debris ingestion, with the greatest risk of high debris loads associated with species that employ surface seizing, diving and filtering foraging strategies, as has been observed in previous studies comparing filter feeding and surface seizing to prey pursuing seabirds [59, 60]. The first case study where a significantly lower sum of debris was observed in the diving (group 3) fluttering shearwater than the surface feeding fairy prion (group 7), with both species inhabiting an overlapping range and exhibiting a similar diet, reinforces the observation of higher debris ingestion in surface feeding birds. This higher risk associated with surface filtering may result from non-food debris filtered from the water along with food during foraging bouts.

Debris ingestion occurred within all diet groups but was most abundant in seabirds with crustacean dominant diets, and least common in birds having fish dominant diets. This demonstrates that crustacean dominant diets are a major risk factor for marine debris. It's possible that there is a dietary resemblance between some small crustaceans and hard plastic marine debris [12], but plastic ingestion while feeding on crustaceans may be accidental as birds feed on pelagic crustaceans floating among plastic. In contrast, floating plastic and other debris does not typically resemble fish either by shape or behaviour, and this is probably why marine debris is not attractive to piscivorous seabirds. These findings reflect early observations of plastic ingestion occurring more commonly among seabirds with crustacean diets and less commonly among predominantly piscivorous seabirds [60].

Encounter density is an important driver of marine debris ingestion, explaining variation in debris ingestion incidence that was not explained by diet, foraging method nor taxa (SI Table 2). The effect of exposure of Procellariiform taxa to regions heavily polluted with marine debris is clear when comparing debris ingestion in polluted and unpolluted regions. In the heavily polluted Hawaiian Islands, seabirds ingest debris at much higher rates than birds from our study. The majority of Hawaiian albatrosses, including Laysan (89.5%) and black-footed albatrosses (58.8%) and gadfly petrels; including Bonin petrel (100%) [61], ingest marine debris, while Australasian albatrosses and gadfly petrels displayed very low incidences of debris ingestion (Figure 3). White-chinned petrels have only 0.9% incidence of debris in Australasia compared to 63.1% off Brazil, a region with much higher rates of plastic pollution [62]. Other studies also highlight environmental exposure to debris as an important factor in incidence of debris ingestion [63]. Van Franeker and Law (2015) highlight that fulmarine petrels that forage exclusively within relatively unpolluted Antarctic seasonal sea ice zone do not ingest plastic, but those that winter outside this region ingest plastic on these trips[63]. As many seabirds have a broad distribution and exact foraging routes of individuals are not known, the encounter density variable represents a range average and cannot capture local spatial or temporal effects. While encounter density provides an average snapshot of encountered marine debris in a species range, it does not account for specific breeding/wintering distributions, temporal offload of debris during chick rearing, nor trends of individual birds which forage in areas of locally high or low marine debris. Modelling accuracy could be improved with the inclusion of seabird population specific tracking data, local background debris availability data, and better representation of species with small sample sizes.

In general, closely related species share common foraging strategies and diets (SI Table 1)[16], and we propose that taxonomic grouping captures much of the diet and foraging information for many species. Storm petrels were the taxonomic group with the highest incidence of marine debris ingestion. Though their diet and foraging groupings support a moderate risk of debris ingestion, storm petrels forage exclusively at the surface and have a crustacean-dominant diet, two high-risk factors for debris ingestion. High incidences of debris ingestion in storm petrels have been recorded in the literature in multiple ocean basins, including 100% of Tristram's Storm-petrel, *Oceanodroma tristrami*, chicks collected in Hawaiian islands [64] and 79% of white-faced storm-petrel remains in gull pellets collected in the North Atlantic [65]. A high debris encounter density likely also drives this high debris ingestion incidence pattern in storm petrels, though there may be additional unknown factors. With this evidence of high debris ingestion in multiple storm petrel species across multiple oceans, storm petrels are one of the taxonomic groups at very high risk of debris ingestion globally.

Giant petrels presented the next highest incidence of debris ingestion, though placed in low-moderate risk foraging and diet groups and with low-moderate encounter density. Marine debris has been found in 72.7% of southern giant petrel pellets in Patagonia [66].Giant petrels' habit for scavenging and predating on smaller plastic-ingesting seabirds [66] are a likely source of risk unique to this taxa. In this study we encountered secondary ingestion of marine debris in a giant petrel that had eaten a shearwater with ingested plastic shortly before death.

It is interesting to note that the AIC of the best model to explain the number of debris items ingested by a seabird was equivalent to a best model also including the presence/absence of a restricted isthmus juncture in the gut. The albatross taxonomic group is the only taxonomic group lacking this isthmus juncture, a gut structure that puts Procellariiformes at increased risk of retaining ingested plastic [20]. As albatross are the only bird group lacking an isthmus juncture, this is the reason why isthmus juncture presence does not add to the best model as this information is already equivalently captured by the albatross bird group. The lack of isthmus juncture in albatrosses may also reduce detection of marine debris ingestion by albatrosses, compared to other taxonomic groups, if albatrosses regurgitate ingested marine debris before death.

Rarely do studies report on the same species in two geographically disparate locations. We were able, however, to provide evidence of the effect of marine debris pollution in foraging ranges of two geographically separate populations of a single species, flesh-footed shearwater [57, 58], removed from the confounding effects of differing diet, foraging behaviour and taxonomy. Flesh-footed shearwaters are widely distributed across the Indian and Pacific Oceans, and often solitary when foraging. Their movements cover a broad pelagic distribution, and individual flight paths vary considerably; with birds from different breeding colonies undertake markedly different migratory routes [57, 58]. Tracks of individuals from the eastern Pacific flesh-footed shearwater populations show they migrate to the polluted north-west Pacific Ocean [57]. Tracking of the Indian (western) population shows that they migrate to the less polluted south-eastern Indian ocean [58]. The pattern of debris ingestion in eastern compared to western flesh-footed shearwaters reflects the debris they are likely to encounter in their respective foraging ranges, with significantly higher average debris ingestion in eastern birds (Figure 5). The variability, including individuals with very high incidence of debris ingestion likely reflects the foraging tracks of individual birds into variably polluted regions of the Pacific Ocean.

Evidence for the influence of marine debris pollution in foraging ranges is also demonstrated by comparing species with known foraging paths or restricted ranges. Fluttering shearwaters feed in the relatively unpolluted Tasman Sea [15]. The average fluttering shearwater does not ingest marine debris, and the highest number of recorded items ingested was 3, demonstrating that debris ingestion is rare in species restricted to low pollution habitat. In contrast, short-tailed shearwaters follow an annual migration route passing through heavily polluted parts of the Northern Pacific Ocean during the Northern hemisphere summer and returning to the lesser polluted Southern Pacific and Southern Ocean during the Southern hemisphere summer [15]. The median short-tailed shearwater we collected ingested 4 debris items with a maximum of 27. Short-tailed shearwaters collected in the north Pacific Ocean in June-July (n=87) contain a mean of 15.1±2.9 marine debris items [31], while those collected in the Southern Pacific Ocean in November-December for in this study, following their return migration (n=201), contained a mean of 4.43±4.17 marine debris items. Assuming marine debris concentrations in shearwater foraging habitat remain comparable between the studies, this marked decline in ingested debris as this species migrated from heavily to marginally polluted foraging areas, adds to the evidence that exposure to marine debris is an important driver in marine debris ingestion.

Combining the results of ecological factors that contribute to increased debris ingestion in Procellariiform seabirds, we predict that storm petrels (*Hydrobatidae* and *Oceanitidae*) are the bird group currently at the greatest risk of a high incidence of debris ingestion due to satisfying this combination of ecological factors. We suggest that prions and fulmarine petrels display behavioural and dietary risk factors of high debris ingestion, though these bird groups largely inhabit the lesser polluted Southern Ocean. We predict that prions and fulmarine petrels are the groups most vulnerable to amplified debris ingestion if there is a future increase of plastic pollution in the Southern Ocean. We predict increased incidences of debris ingestion, and resulting harm, would occur across all Procellariiform species should their current level of environmental exposure to debris increase. Conversely, reducing environmental exposure of seabirds to debris, by reducing oceanic debris inputs or removing extant debris, should reduce the incidence of debris ingestion in seabirds.

In summary, there is now strong support that the seabird species at greatest risk of debris ingestion can be forecasted by examining their ecology. Among the ecological drivers of variability in Procellariiform debris ingestion, the combination of taxonomy, foraging method, diet, and exposure to marine debris pollution are the most central factors driving incidence of marine debris ingestion. Expanding on our empirical results and using species distributions and marine debris density estimates in the ocean, we forecast that the species most at risk of ingesting debris forage at the surface by surface seizing, diving and filtering, have a crustacean dominant diet, and feed in or near marine debris hotspots.

Figures

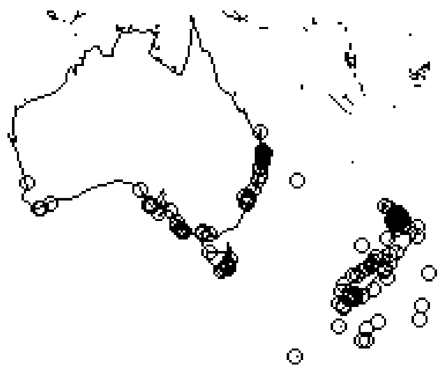


Figure 1. Locations of seabird carcass collection across Australia and New Zealand, including oceanic by-catch locations.

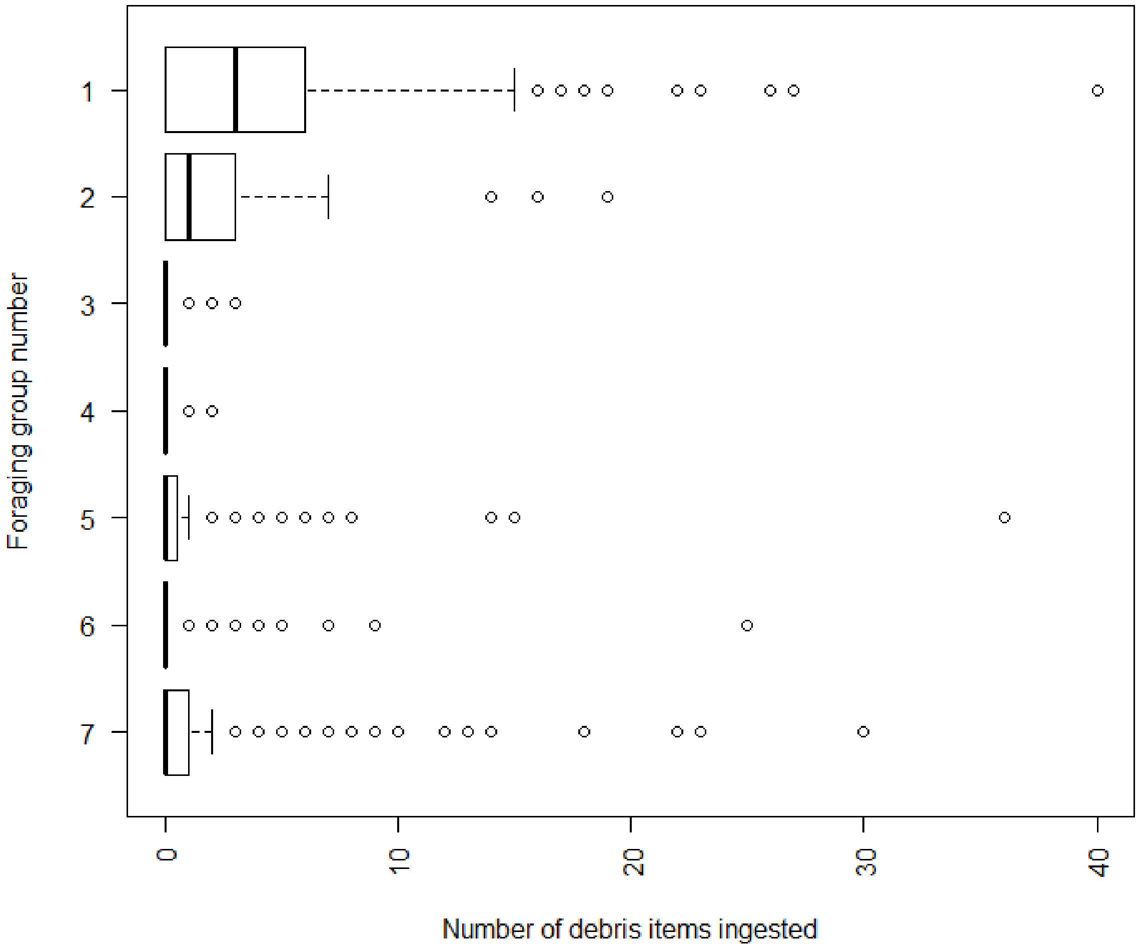


Figure 2. Box-plot of number of marine debris ingested by seven foraging groups determined by cluster analysis of seabird foraging behaviour following Marchant and Higgins (1990). Group 1: mostly surface diving and pursuit diving/plunging. Group 2: mixed surface foraging including filtering, also seizing, plunging and dipping. Group 3: feeding under the surface by pursuit diving/plunging. Group 4: surface seizing and diving. Group 5: mostly pursuit plunging with some surface and pursuit diving. Group 6: surface seizing and plunging with minimal other feeding methods. Group 7: surface seizing, pattering and dipping.

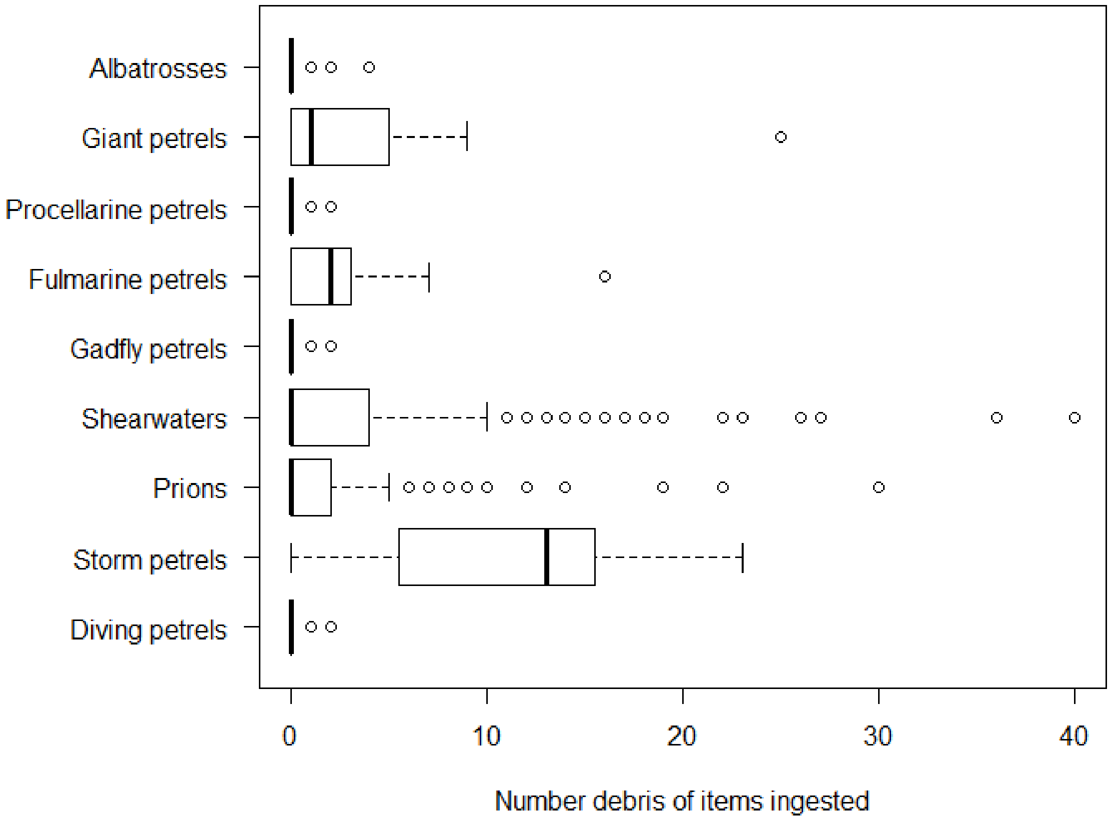


Figure 3. Box-plot of number of marine debris ingested by Procellariiform taxonomic groupings.

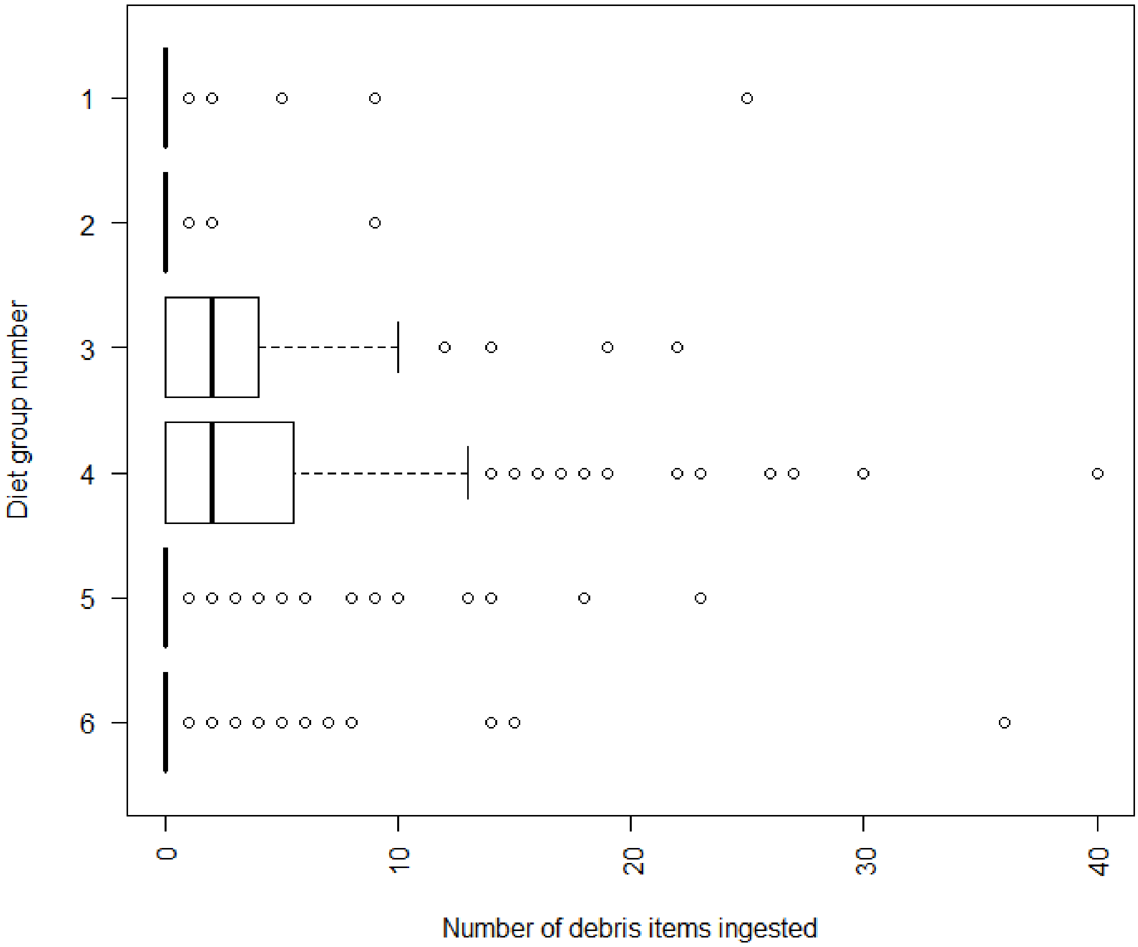


Figure 4. Box-plot of number of marine debris ingested by six diet groups determined by cluster analysis of seabird primary diets following Marchant and Higgins (1990). Group 1: diet squid dominant, with some fish, crustaceans and scavenging. Group 2: diet fish dominant, but some squid and crustaceans. Group 3: diet crustacean dominant but may take fish and squid. Group 4: diet cephalopods and crustaceans dominant, with some also taking fish. Group 5: diet mostly fish and crustaceans. Group 6: diet mostly fish and squid with some scavenging.

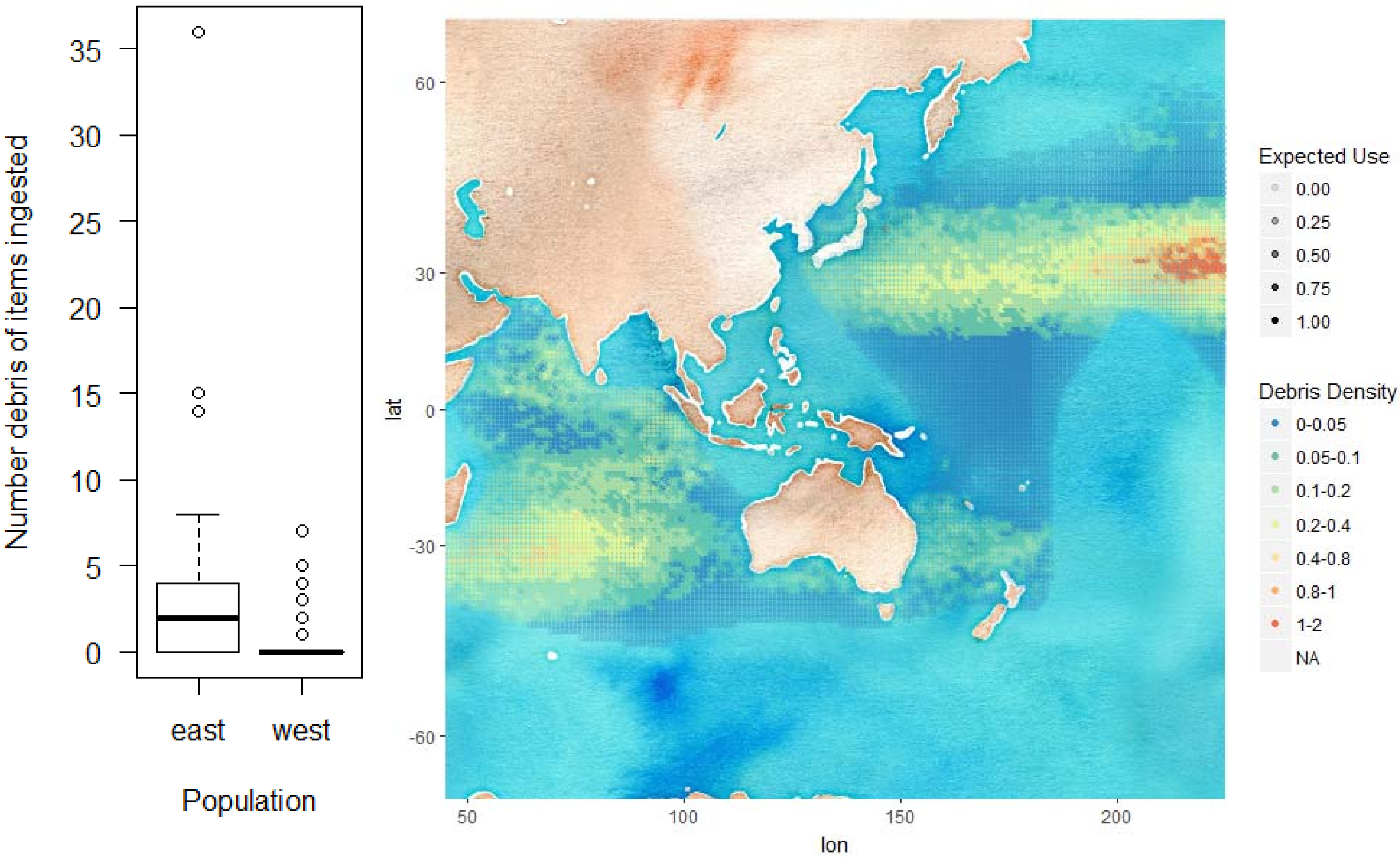


Figure 5. Box-plot (left) of number of marine debris ingested by flesh-footed shearwater, *Ardenna carneipes,* populations foraging to the east of longitude 145, and west of longitude 145. Map (right) of density of marine debris within the foraging range of flesh-footed shearwaters. Seabird species distribution and expected habitat use data were sourced from BirdLife International's seabird database [2]

Supplementary information

Table 1: Incidence of plastic ingestion in Australasian seabird species. N is the number of individuals that were examined for debris ingestion. Percept debris ingestion is the percentage of the individuals sampled that had ingested debris. Median, standard deviation and maximum number of debris items ingested for each species. Diet clusters included Group 1: diet squid dominant, with some fish, crustaceans and scavenging. Group 2: diet fish dominant, but some squid and crustaceans. Group 3: diet crustacean dominant but may take fish and squid. Group 4: diet cephalopods and crustaceans dominant, with some also taking fish. Group 5: diet mostly fish and crustaceans. Group 6: diet mostly fish and squid with some scavenging. Foraging clusters included Group 1: mostly surface diving and pursuit diving/plunging. Group 2: mixed surface foraging including filtering, also seizing, plunging and dipping. Group 3: feeding under the surface by pursuit diving/plunging. Group 4: surface seizing and diving. Group 5: mostly pursuit plunging with some surface and pursuit diving. Group 6: surface seizing and plunging with minimal other feeding methods. Group 7: surface seizing, pattering and dipping. Encounter density is the sum of the oceanic debris density across the birds range as per Wilcox (2015), multiplied by the species' expected use of each area as per BirdLife international's seabird database [2].

Species	n	Percent debris ingestion	Median	Standard deviation	Max	Diet cluster	Foraging cluster	Encounter density
Diomedeidae- Albatrosses								
Albatrosses	263	0.02	0	0.3	4			
Antipodean albatross	1	0	0	NA	0	6	6	11.09
Black-browed albatross	9	11.1	0	0.3	1	2	6	155.9
Buller's albatross	90	1.1	0	0.2	2	1	6	5.77
Campbell albatross	4	0	0	0	0	2	6	16.24
Chatham Island albatross	1	0	0	NA	0	6	6	90.48
Gibson's albatross	2	0	0	0	0	6	6	11.09

Grey-headed albatross	4	25	0	2	4	6	6	26.25
Light-mantled sooty albatross	6	16.7	0	0.4	1	1	6	1.8
New Zealand white-capped albatross	85	0	0	0	0	6	6	141.15
Salvin's albatross	30	0	0	0	0	6	6	59.96
Shy albatross	26	3.8	0	0.2	1	6	6	141.15
Sooty albatross	1	0	0	NA	0	4	6	125.68
Southern royal albatross	2	0	0	0	0	1	6	11.56
Wandering albatross	5	0	0	0	0	6	6	145.19
Procellariidae- Petrels, Shearwaters and Prions								
Procellarine petrels	252	0.02	0	0.2	2			
Black petrel	7	0	0	0	0	2	6	9
Grey petrel	7	0	0	0	0	4	6	60.02
Tahiti petrel	1	0	0	NA	0	NA	NA	13
Westland petrel	16	12.5	0	0.5	2	2	4	2.97
White-chinned petrel	221	0.9	0	0.1	1	2	4	95.36
Fulmarine petrels	12	58.3	2	4.6	16			
Antarctic petrel	1	100	2	NA	2	4	6	5.6
Cape petrel	7	42.9	0	5.9	16	4	2	167.44
Southern fulmar	4	75	3	2.9	7	4	6	87.65
Giant petrels	12	58.3	1	7.5	25			
Northern giant petrel	4	50	0.5	2.4	5	1	6	87.03

Southern giant petrel	8	62.5	0	9.2	25	1	6	73.9
Gadfly petrels	37	19	0	0.53	2			
Black-winged petrel	2	0	0	0	0	3	7	108.8
Cook's petrel	7	14.3	0	0.76	2	1	NA	223.78
Gould's petrel	5	60	0.5	1	2	NA	NA	23.06
Great-winged petrel	4	0	0	0	0	1	7	86.68
Grey-faced petrel	4	0	0	0	0	1	7	86.68
Kerguelen petrel	3	33.3	0	0.58	1	4	6	37.71
Mottled petrel	3	33.3	0	0.6	1	1	6	243.38
Petrel (unknown sp)	1	0	0	NA	0	NA	NA	NA
Providence petrel	1	0	0	NA	0	NA	NA	155.38
White-headed petrel	6	16.7	0	1	NA	4	6	19.08
White-necked petrel	1	0	0	NA	0	6	7	101.93
Shearwaters	744	50.3	0	4.5	40			
Flesh-footed shearwater	213	25.8	0	3.2	36	6	5	398.14
Fluttering shearwater	70	10	0	0.5	3	5	3	15.38
Hutton's shearwater	4	0	0	0	0	5	3	8.75
Little shearwater	8	50	0.5	1.4	3	NA	NA	139.9
Short-tailed shearwater	332	86.4	4	4.8	27	4	1	292.2
Sooty shearwater	89	21.3	0	5.3	40	4	1	380.58
Wedge-tailed shearwater	28	14.3	0	1.7	9	2	6	120.02
Prions	372	38.4	0	3.1	30			

Antarctic prion	17	70.6	1.5	4.8	19	3	2	85.2
Blue petrel	2	50	15	21.2	30	4	7	37.56
Broad-billed prion	14	21.4	0	1.5	5	4	2	167.35
Fairy prion	236	25.8	0	1.6	14	5	7	46.26
Salvin's prion	24	70.8	2	3.3	14	3	2	NA
Slender-billed prion	79	60.8	1	3.6	22	3	7	28.84
Hydrobatidae – Storm petrels								
Storm petrels	7	85.7	13	8.11	23			
Black-bellied storm petrel	1	0	0	NA	0	6	7	503.68
White-faced storm petrel	6	100	13	7.1	23	5	7	290.7
Pelecanoididae- Diving petrels								
Diving petrels	32	6.3	0	0.4	2			
Common diving petrel	31	6.5	0	0.4	2	5	3	18.62
South Georgian diving petrel	1	0	0	NA	0	1	3	0.30

Table 2: The best model of ecological variables used to explain how much marine debris is ingested by seabirds includes taxonomic grouping, foraging grouping, diet grouping and density of marine debris encountered in the foraging range. The model explains 48.9% of the variance in marine debris ingested by seabirds in our study.

Parametric coefficients:

Ecological Variables	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**	
Foraging 7 (Intercept)	7.308e-01	1.962e+00	0.372	0.7096	
Foraging 6	-1.615e+00	9.311e-01	-1.734	0.0831	.
Foraging 5	4.091e+00	1.773e+00	2.307	0.0212	*
Foraging 4	4.204e+01	2.438e+05	0.000	0.9999	
Foraging 3	-1.177e+00	1.992e+00	-0.591	0.5548	
Foraging 2	-6.869e-01	7.661e-01	-0.897	0.3701	
Foraging 1	3.427e+00	2.225e+00	1.540	0.1237	
Storm petrels	7.308e+00	2.253e+00	3.244	0.0012	**
Prions	1.144e+00	2.183e+00	0.524	0.6002	
Shearwaters	3.851e-01	8.951e-01	0.430	0.6671	
Gadfly petrels	-9.351e-01	2.141e+00	-0.437	0.6623	
Fulmarine petrels	2.203e+00	2.068e+00	1.065	0.2869	
Procellarine petrels	-4.723e+01	2.438e+05	0.000	0.9998	
Giant petrels	3.936e+00	1.690e+00	2.329	0.0200	*
Albatrosses	-1.562e+00	1.571e+00	-0.994	0.3202	
Diet 5	-1.729e+00	1.025e+00	-1.687	0.0919	.
Diet 4	8.874e-01	1.144e+00	0.776	0.4379	
Diet 3	-5.698e-01	1.053e+00	-0.541	0.5887	
Diet 2	1.309e+00	1.347e+00	0.971	0.3315	
Diet 1	-5.997e-01	8.164e-01	-0.735	0.4627	
Encounter density	-1.314e-02	1.941e-03	-6.769	1.81e-11	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Chapter 3: How much plastic pollution does it take to kill a seabird?

Lauren Roman[1], Britta Denise Hardesty[2], Mark A. Hindell[1,3], Chris Wilcox[2]

1 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia,

2 CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia,

3. Antarctic Climate and Ecosystems CRC, University of Tasmania, Hobart, Tasmania, Australia,

Abstract

Procellariiformes are the most threatened bird group globally, and the group with the highest frequency of marine debris ingestion. Marine debris ingestion is a globally recognized threat to marine biodiversity, yet the relationship between how much debris a bird ingests and mortality remains poorly understood. Using cause of death data from 1733 seabirds of 51 species, we demonstrate a significant relationship between ingested debris and a debris-ingestion cause of death (dose-response). There is a 20.4% chance of lifetime mortality from ingesting a single debris item, rising to 100% after consuming 93 items. Obstruction of the gastro-intestinal tract is the leading cause of death. Overall, balloons are the highest-risk debris item; 32 times more likely to result in death than ingesting hard plastic. These findings have significant implications for quantifying seabird mortality due to debris ingestion and provide identifiable policy targets aimed to reduce mortality for threatened species worldwide.

Introduction

Pollution of the world’s oceans by anthropogenic marine debris is a global problem [8]. With 250 000 tonnes of marine debris afloat currently, our mismanaged waste presents a ubiquitous threat to marine wildlife [8]. Ingestion of buoyant marine debris in the ocean is a widespread, emerging threat to seabirds [7, 10], particularly so for albatrosses and petrels (Procellariiformes) [12], that mistake the floating trash for food [11, 12]. Seabirds are the world's most threatened group of birds, with nearly half of species experiencing population declines, and 28% threatened globally [67]. Presently, half of the world's seabird species ingest marine debris [51], with the greatest expected adverse effects occurring in Australasia, at the Southern Ocean boundary of the Tasman Sea [1] where the highest global seabird biodiversity occurs [17].

Significant declines in Australasia's albatross and petrel populations are driven by a number of threats [68], but the contribution of marine debris ingestion is unknown. At least 51 of Australasia's Procellariiform species ingest marine debris (Chapter 2), likely more species ingest marine debris, although it has not been documented [68]. Ninety nine percent of all seabird species are predicted to ingest marine debris by 2050 [1]. The ubiquity of debris ingestion among threatened and declining seabirds highlights the need to quantify the level of threat that it poses to seabirds. Quantifying the effects of the ingestion of marine debris on individual mortality, and ultimately on wild populations, is one of the primary research priorities in marine debris research [50].

Despite observations of seabird mortalities resulting from ingesting debris [21], and anecdotal evidence that ingestion has sublethal and lethal impacts on seabirds, a quantitative relationship is yet to be established. This is due to the difficulty of establishing a dose-response relationship between ingestion and mortality. In the absence of experimental feeding trials, the necropsy of wild seabirds collected deceased can provide data to estimate a dose-response relationship. Wild seabirds die for many reasons including starvation, disease, injury, fisheries by-catch, and the ingestion of marine debris. We used seabirds that had an identifiable cause of death (*e.g.* fisheries, by-catch or advanced disease) as a control group (assuming their death was random with respect to the ingestion of marine debris). We compared these birds that died of indeterminate causes to those that were identified as having died due to marine debris ingestion (gut blockage, perforation or impaction). With this information, we investigated whether the ingested debris load is lowest in seabirds dying due to non-marine debris related causes, increasing for seabirds with indeterminate causes of death (which could potentially have died due to debris ingestion), and highest in seabirds that died from ingesting marine debris. Ultimately, we also used all seabirds in the dataset to estimate the relationship between the probability of death due to marine ingestion and the load of ingested marine debris. We aim to determine whether there is a dose-response relationship between marine debris ingestion and seabird mortality, and if so, to predict the relationship between the load of ingested marine debris and the probability of death due to marine debris ingestion.

Methods

Material collection:

Data for this study included deceased seabirds obtained as fisheries by-catch, veterinary casualties and beach-washed carcasses collected from Australia and New Zealand. In Australia, Fraser Island, QLD, was the most northerly collection point, and Macquarie Island the most southerly. Collection spanned the width of the country from Ballina, NSW in the east and Perth, WA in the West. Collection in New Zealand spanned the country North (Bay of Plenty) to South (Invercargill area) and included by-catch in oceanic regions between the south of the continent and the Auckland Islands to the south.

In total, 1733 individuals of 51 species were collected and necropsied following Van Franeker's collection and dissection procedures [55], and cause of death was determined. Birds with a cause of death unrelated to the ingestion of marine debris (fisheries by-catch, some veterinary casualties, injury, disease/infection) were assigned the category "non-debris" cause of death. Seabirds with ingestion of marine debris resulting in clear gut impaction, perforation or blockage, (often with associated local signs of infection and irritation and/or undigested food blocked from passing through the gut), were assigned to category "debris" cause of death. In these cases, the object responsible for the death was determined by its association with the site of lesion. Seabirds where debris was present but the cause of death could not be positively determined were assigned an 'indeterminant' cause of death. When gut blockage or impaction was suspected but could not be positively confirmed as the cause of death, the cause of death was also ruled as indeterminate. As a result, the birds deemed as having died from ingested marine debris is conservative and likely lower than the actual number.

Marine debris items removed from the seabirds were rinsed, dried and weighed. For rigid objects (mostly fragments of hard marine debris), the length, width and depth were measured at the longest edge. Volumes were calculated by multiplying the weight by an average density value for the material type; 0.95g per cm^3 for hard plastics [69, 70], by 0.91 g per cm^3 for balloons [71] and 7.7g per cm^3 for fishing hooks [72]. The volume of other items, including large quantities of soft plastic, rubbers and expanded foams, were measured per 1ml of water displacement.

<u>Data preparation</u>

To examine the dose-response relationship between marine debris and seabird death, we assigned each seabird death into three cause of death categories, as described above: 1) known, non-debris ingestion related (*KND*), where there was a clearly identifiable cause such as drowning as fisheries by-catch; 2) indeterminate (*Ind*), where there was marine debris present in the gut but also other possible causes, such as starvation and 3) known, marine debris ingestion related (*KD*), where there was a gut blockage, or other strong evidence of the ingested debris being the cause of mortality. If debris ingestion results in death, we expect that the number of ingested debris items ingested should scale as: KND < Ind < KD. This slope of the proposed relationship is shown in Figure 2, and is adapted from Wilcox et al.

<u>Statistical Analysis</u>

Statistical analyses were performed using R (Version 3.3.3)[56], following Wilcox et al. [73]. We tested for variation in the count of ingested marine debris items present in individual birds within the three cause of death categories using a generalized linear regression model (GLM), using a negative binomial error, due to over-dispersion in the data, which proved adequate based on a Chi square test. In addition to cause of death, we included species, age, average species weight and taxonomic family variables, as these can influence the frequency of debris ingestion [73]. We selected the best model using the Akaike Information Criterion (AIC) (Table 1), which was used to estimate the pairwise differences between the coefficients for cause of death to determine whether they followed the expected KND < Ind < KD order (Figure 2).

An interval value was assigned for the probability of death due to marine debris ingestion for each bird. Birds with known causes of death other than debris ingestion (KND) were assigned [0,0], birds with deaths caused by debris ingestion (KD) were assigned [1,1]. Birds with indeterminate causes of death were assigned the range [0,1]. We performed a logistic regression to relate the probability of death due to debris ingestion to the number of debris items in an animal's gut. A Monte Carlo technique was used to accommodate the interval values for indeterminate causes of death, randomly drawing a value in the interval [0,1] for each bird in the indeterminate cause of death category to fit the model to the full dataset across all three causes of death. We captured the estimated coefficient for the number of marine debris items in the gut and its standard error. This process was repeated 1,000 times. The output coefficient and its significance for each fit was used for each iteration to create an expected distribution. We accounted for the effect of gut volume on the relationship between chance of death due to marine debris and the number of items by including the average species weight of the bird, as per Marchant and Higgins [16] as a covariate, on the assumption that species weight is proportional to gut volume.

Results

Of the 1733 seabirds examined, 558 (32.2%) had ingested marine debris, ranging from 1-40 items, with a maximum debris weight of 3440mg and volume of 3621mm^3. In total, 2671 items were collected. Hard plastic, both fragments and pellets, were most common, accounting for 92.4% of all items ingested. The remaining items ingested included soft plastics such as packaging (2.1%), balloon fragments (2%), rubbers and foams including polystyrene, expanded polyethylene and other synthetic foams (1.3%), rope and rope fibres (1%), fishing related rubbish (0.7%) and other debris types 0.5%. Weight and volume of ingested debris was not recorded for 27 of the samples. The type of debris ingested was not recorded for 17 of the samples.

The cause of death of 1265 (73%) of the seabirds that could be determined was not debris ingestion (KND). Thirteen birds died as a result of marine debris ingestion (KD); five fairy prions, *Pachyptila turtur*, four short-tailed shearwaters, *Ardenna tenuirostris*, one Salvin's prion, *Pachyptila salvini*, one Antarctic prion, *Pachyptila desolata*, one blue petrel, *Halobaena caerulea,* and one light-mantled sooty albatross, *Phoebetria palpebrata*.

Blockage of the gastrointestinal tract was the leading cause of mortality (7 birds), followed by obstruction of the gastrointestinal tract (5 birds) causing infection or other complications, and one perforated gut. The site of blockage and obstruction was the isthmus between the proventriculus and the gizzard in 8 birds, the gizzard in two birds and the entrance to the small intestine in two birds. The perforation of the gut occurred in the proventriculus. The items causing death were hard plastics (n=7), balloons (n=3) and expanded foam (n=2). The gut perforation was caused by plastic strapping. A further 9 birds; four short-tailed shearwaters, two slender-billed prions, *Pachyptila belcheri*, one Salvin's prion, one white-faced storm-petrel, *Pelagodroma marina*, and one southern fulmar, *Fulmarus glacialoides*, were deemed very likely to have died from marine debris due to obstruction or blockage of the GI tract, but this could not be confirmed due to the decomposition of the bird. The obstructing items were hard plastic (n=4), balloons (n=2), soft plastic packaging (n=2) and synthetic rope (n=1). These birds were allocated an indeterminate cause of death for the purpose of this model. The remaining 446 birds were allocated an indeterminate cause of death (Ind). The number of items ingested by seabirds ranked according to the cause of death was: Known (non-marine debris related) < Indeterminate (possibly marine debris related) < known (marine debris related) (Figure 1), following the theoretical model (Figure 2). Seabirds that died from marine debris had significantly higher numbers of ingested marine debris than birds with indeterminate causes of death and those known to have died from other causes ($P<0.05$).

The best model, based on AIC, for the relationship between the number of debris items in the gut and the cause of death includes a main effect for species and the species weight (Table 1), having an AIC of 3757.7.

The relationship between the number of ingested items and the probability of death due to debris ingestion had a significantly positive slope term (Figure 3). Using the median values of the regression parameters from the Monte Carlo analysis, and the average species weight, we were able to predict the relationship between the load of ingested marine debris and the probability of death due to marine debris ingestion (Figure 4). Species was not included in this model as our data did not include KND, Ind and KD individuals across all species. Our model shows that a bird with one ingested debris item has a 20.4% chance of mortality, rising through 50% chance of mortality at 9 items and 100% at 93 items. Using the bounds of this relationship across all estimated values from our Monte Carlo simulations, we found a relatively small amount of uncertainty (Figure 4).

Discussion

Marine debris load appears to explain risk of death resulting from debris ingestion. Debris loads were lowest in seabirds dying due to non-marine debris related causes, rising through indeterminate causes of death and was highest in seabirds that died from marine debris ingestion. Seabirds that died of debris ingestion had, on average, greater marine debris loads in their gastro-intestinal tracts. This supports similar findings in sea turtles that the causes of death are segregated in terms of plastic concentration [73]. Secondly, when we modelled the probability of death due to marine debris ingestion, we found a positive relationship, confirming that larger loads of marine debris items in the gastro-intestinal tract lead to a higher probability of mortality. In this study each seabirds' mortality and ingested debris load observed over an unknown period of its lifetime. We do not know either the birds age or turnover rate for the ingested debris loads, and assume the debris load we recorded at death represents an average load and turnover that lead to the death for the bird. These results can be used to predict the lifetime mortality rate for seabirds where the load of marine debris items is known. It is interesting to note that a bird has a 20.4% chance of mortality with a single ingested marine debris item, a statistic supported by two individuals in our study having died as a result of having ingested a single item causing obstruction. In one case of mortality, a knotted balloon obstructed the entrance to the intestine, and in the second, a hard plastic blockage in the isthmus juncture. This lifetime mortality model is limited by the small number of KD samples relative to each KND and Ind samples, and this study would be improved with the addition of further KD samples.

Some Procellariiform species, including short-tailed shearwater, slender-billed prion, Salvin's prion and white-faced storm petrels have a median debris ingestion of 1 or greater (Chapter 2), and this model provides a valuable quantitative framework to modelling population impacts on Procellariiform seabirds, especially species with high incidences of marine debris ingestion (Chapter 2).

All but one death resulted from obstruction in one of three locations; the gizzard and the small junctures leading in and out of the gizzard, causing either a large obstruction through to a complete blockage. The unique gizzard morphology of Procellariiform seabirds, (the gizzard is separated from the proventriculus by an isthmus juncture where hard items can become lodged and not easily regurgitated) puts Procellariiform birds at higher risks of obstructions (Chapter 2). For this reason we can expect Procellariiformes to be at higher risk of mortality from marine debris than other species.

The composition of the marine debris influenced the probability of the likelihood of mortality, as observed in sea turtles. In turtles small hard plastic fragments may pass quickly through the gut with little incident, but soft plastics are more likely to compact and contribute to a blockage or obstruction [73]. In seabirds not all ingested debris posed an equal risk of mortality. Hard plastics were responsible for only half of known and probable seabird deaths but were the vast majority of items ingested. Soft plastic packaging, balloon fragments, rubbers and synthetic foams together accounted for only 5.4% of items but were responsible for 42% of probable and known mortalities. The ingestion of a soft item is 14 times more likely to result in death than the ingestion of a hard item. This may be due to soft and pliable items resisting peristalsis and becoming obstructions [74]. Obstructions of soft pliable synthetics, including plastics and rubbers, have been recorded in a number of species including dogs [75], cattle [74] and birds [76]. In birds, such obstructions can cause death by enteritis, as well as blocking the passage of food, which results in starvation [76].

Balloons were the marine debris most likely to cause mortality. Where ingestion of balloons or balloon fragments were found, these fragments were the known or probable cause of death in 22.2% of balloon ingesting individual birds, with the ingestion of a balloon or balloon fragment were 32 times more likely to result in death than ingestion of a hard plastic fragment. Other studies have highlighted balloons as a high-risk items for ingestion in other taxa [77, 78]. Of particular concern is that seabirds select for balloons when foraging because of their resemblance to prey, especially squid [12]. All balloons in this study were ingested by seabird species that eat squid, suggesting these species are likely to have higher mortality rates. We suggest that reducing the presence of balloons and balloon fragments in the ocean would directly reduce seabird mortalities resulting from marine debris ingestion and would have eliminated the 23% of confirmed KD deaths in this study for which balloons were cause.

In summary, we provide strong evidence that marine debris ingestion can cause death in seabirds, and that the probability of death increases with the number of items ingested. This finding has substantial transboundary implications for managing wildlife population declines due to marine debris ingestion. We propose that the most immediate solution to reduce seabird mortality from anthropogenic marine debris ingestion would be to reduce the number of balloons entering the ocean. In addition, we highlight high-risk marine debris items, providing identifiable policy targets aimed at reducing mortality caused by these debris items. Reducing the input of waste into the environment, in particular high-risk items, will undoubtedly reduce debris ingestion mortality in marine wildlife.

Figures

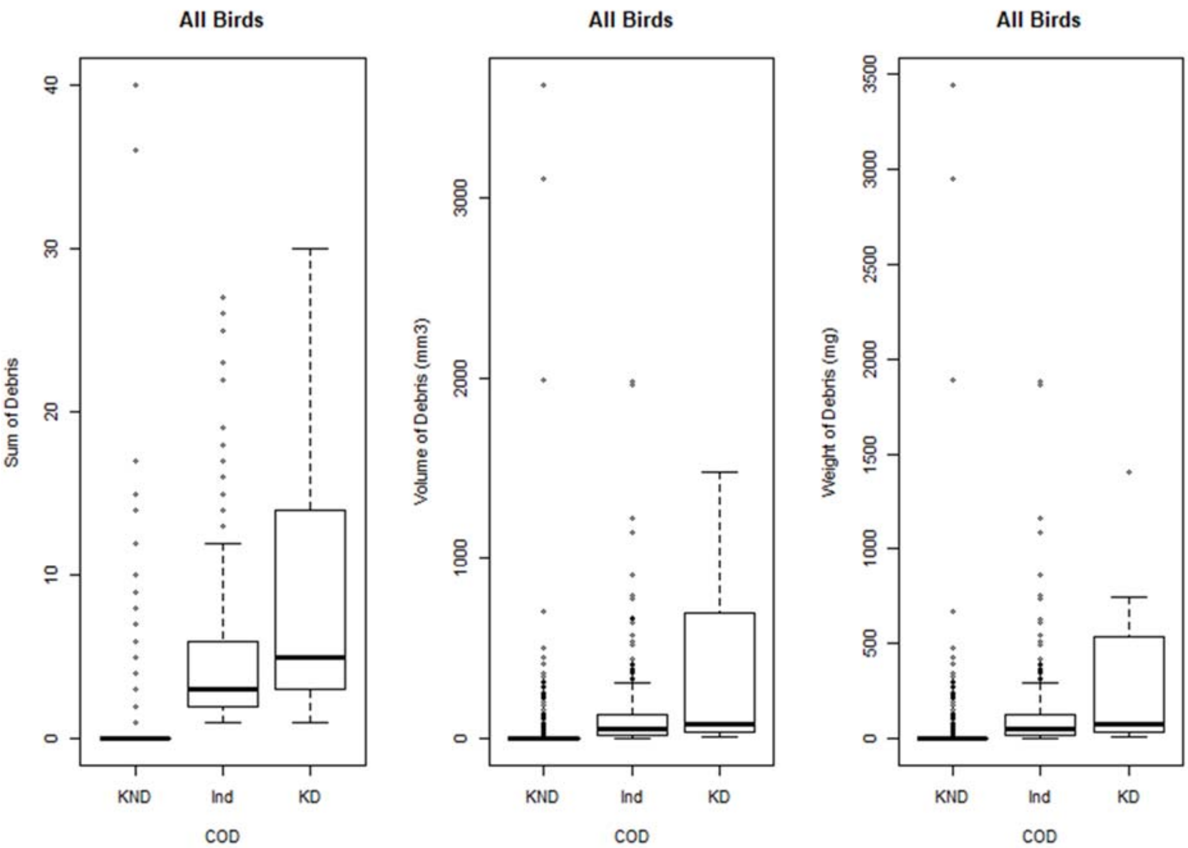


Figure 1. Quantity of marine debris ingested by seabirds by cause of death, showing the median (middle bar) and interquartile range (IQR). The sum (left), cumulative weight (middle), and volume (right) of marine debris items ingested by Procellariiformes that have

died from non-debris (KND) causes, indeterminate (Ind) causes, and marine debris causes (KD). An analysis of variance (ANOVA) and post-hoc pairwise t-test demonstrate that the sum of debris, volume of debris and weight of debris differ significantly between KND, Ind and KD birds.

Table 1. AIC table of explanatory factors driving quantity of ingested debris. AIC table of models run to explain the sum of debris items ingested with cause of death (COD) and individual factors such as species, age, family and average species weight. Note that all models are an improvement over the null model (model 11), with COD, species and species weight as the best model.

Models	AICs	AICs model
1	COD + species + species weight	3757.7
2	COD + species	3759.7
3	COD + age + species	3768.1
4	COD + age + family + species weight	4140.8
5	COC + age + family	4142.1
6	COD + family + species weight	4142.3
7	COD + family	4145.7
8	COD + species weight	4235.1
9	COD + age	4275.4

10	COD	4277.4
11	Null	4918.2

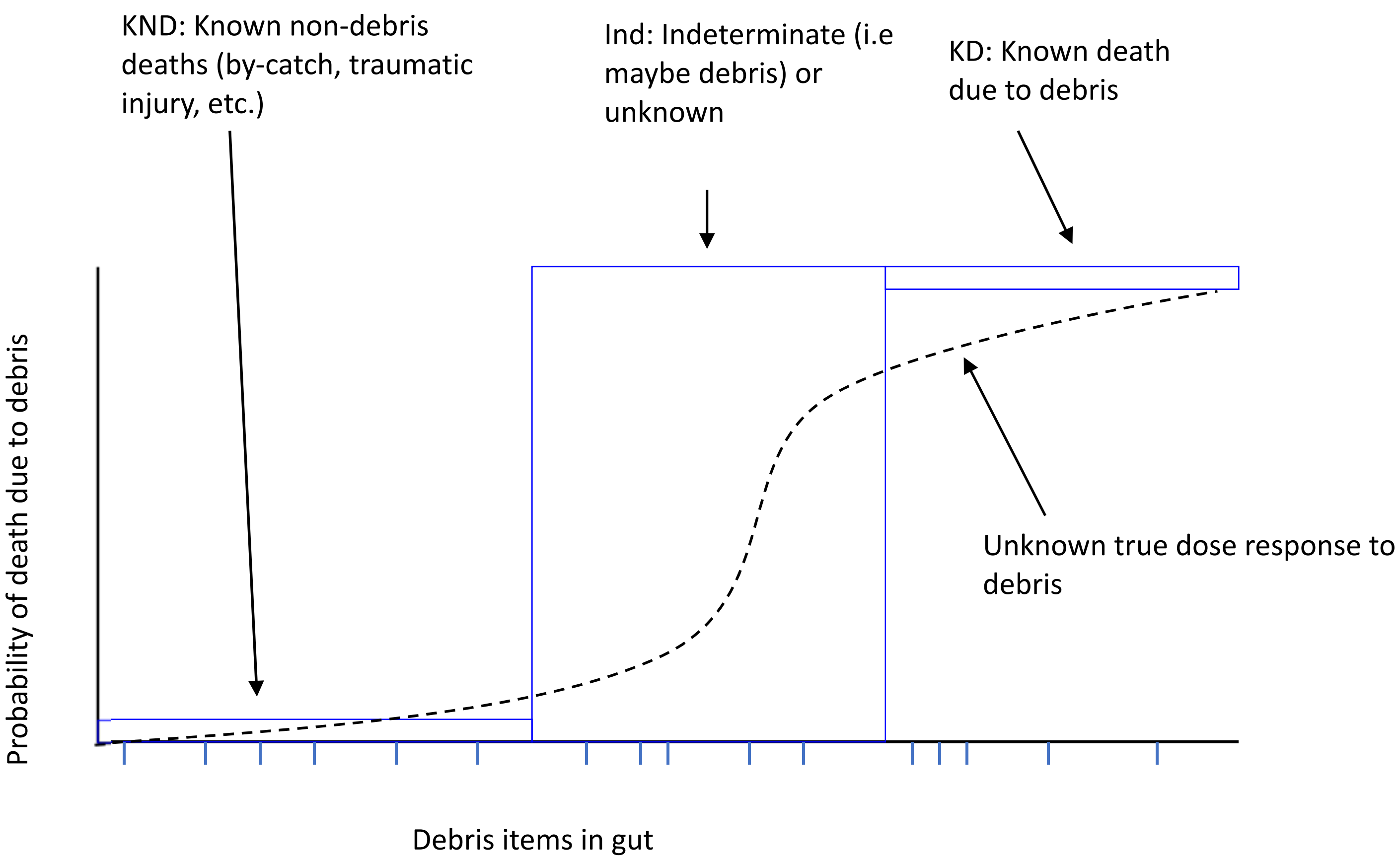


Figure 2. Theoretical dose-response relationship between marine debris and seabird death. This model is adapted from Wilcox et al. 2018 [73]. We assigned each seabird death into three cause of death categories: 1) known, non-debris ingestion related (KND), where there was a clearly identifiable cause such as drowning as fisheries by-catch; 2) indeterminate (Ind), where there was marine debris present in the gut but death may have been due to other causes, such as starvation and 3) known, marine debris ingestion related (KD), where there was a gut blockage, or other strong evidence of the ingested debris being the cause of mortality.

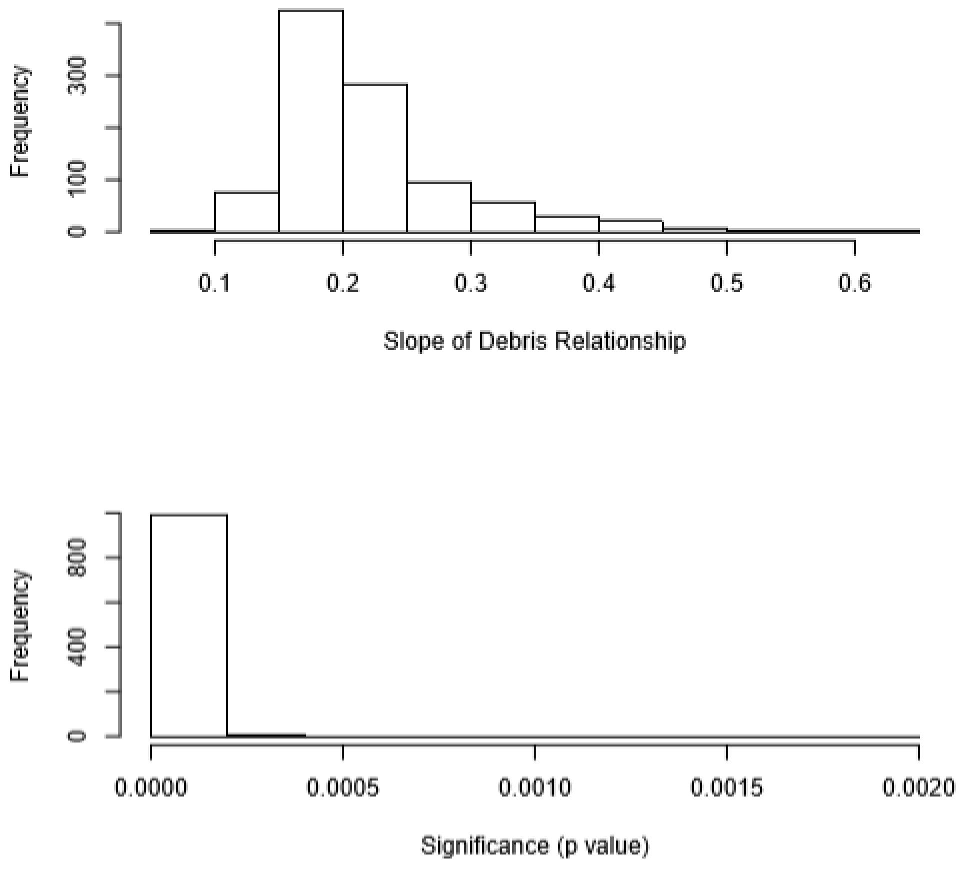


Figure 3. Slope of the relationship between probability of death due to marine debris ingestion and the debris load in the seabird. The top plot shows the distribution of slope

estimates for the number of debris items in the gut, the lower plot shows the significance of these coefficients, from 1,000 Monte Carlo regression analysis samples.

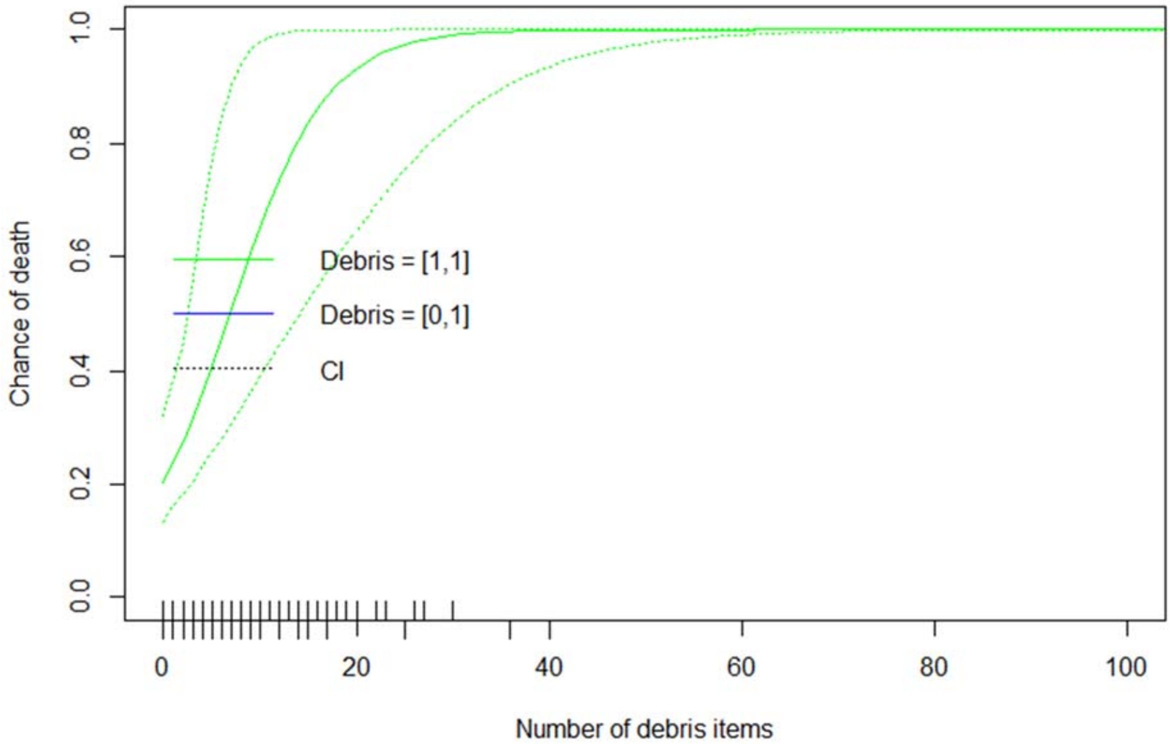


Figure 4. Probability of mortality (chance of death) due to marine debris ingestion with increasing ingested marine debris load. Model results are based on the seabird species weight. Two models are shown, one based on Monte Carlo simulations. The first model assumes the cause of death has been assigned correctly, leading to animals with plastic ingestion as an assigned cause having a probability in the interval [1,1] in the Monte Carlo process. The second model assumes plastic has been assigned incorrectly, leading to a probability in the interval [0,1]. For each model we show the median (solid) and the extreme values (dotted) over 1,000 Monte Carlo simulations. The rug plot along the bottom of the figure shows the number of marine debris items ingested by each of the seabirds in our samples, with top marks indicating seabirds that died of known non-debris ingestion causes, and underneath marks indicating those that died of either debris ingestion or were indeterminate.

Chapter 4: Is plastic ingestion in birds as toxic as we think?

Lauren Roman[1], Linda Lowenstine[2], Laura Maeve Parsley[3,4], Chris Wilcox[5], Britta Denise Hardesty[5], Kirsten Gilardi[6], Mark Hindell[1, 7]

1. Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia,
2. Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California, United States of America,
3. School of Natural Sciences, University of Tasmania, Hobart, Tasmania, Australia,
4. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand,
5. CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia,
6. Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, California, United States of America,
7. Antarctic Climate and Ecosystems CRC, University of Tasmania, Hobart, Tasmania, Australia,

Abstract

Plastic pollution is a modern tragedy of the commons, with hundreds of species affected by society's waste. Birds particularly mistake plastic for prey, and millions of wild birds carry small plastic loads in their stomachs. The big question is how severely the toxicological and endocrine disrupting chemicals in plastic affect an animal's development, reproduction and endocrine function. To address this question, we used multi-generation feeding experiments to test the consequences of plastic ingestion in birds at environmentally relevant loads. Contrary to expectations, we found no evidence of lasting toxicological effects on mortality, adult body weight, hormone levels or reproductive success in birds experimentally fed plastic. However, we found plastic ingestion causes higher frequencies of male reproductive cysts, slower chick growth and delayed sexual maturity, which affects ultimate survival or reproductive output. These findings challenge the routine assumption that birds are harmed by toxicological effects when plastic is ingested.

Introduction

Wildlife ingestion of anthropogenic debris is a global problem and a potentially major threat for all ecosystems [3]. More than 400 avian species occupying a variety of marine and terrestrial ecosystems ingest debris [4], representing many avian families, including seabirds [10], shorebirds [22], and coastal terrestrial birds [12]. This number continues to grow as more species are assessed. In seabirds alone, over 50% of species ingest marine debris, and this has been predicted to rise to 99% of all species by 2050 [10]. In some species, more than 90% of the population ingest plastic, often passing it on to their chicks [12-14, 26]. Considering the frequency of exposure across avian populations, it is probable that hundreds of millions of individual wild birds have ingested plastic.

Plastics introduce an array of chemicals, including endocrine disrupting chemicals, into animals which ingest them [5]. Exposure to endocrine disrupting chemicals has been shown to cause adverse developmental, reproductive, neurological, cardiovascular, metabolic and immune effects, including increased susceptibility to some diseases in mammals [36]. Plastic-associated endocrine disruptors cause altered gene expression [79] and hepatic stress [48] in fish. Plastics in the environment, especially in seawater, undergo further chemical changes through weathering [80] and the uptake of environmental toxins which sorb to the plastics' surface [45]. The toxins from ingested plastics are widely believed to cause adverse health impacts in birds, including endocrine disruption and reduced reproduction [81, 82]; yet despite the high prevalence of ingestion, the toxicological effects of ingesting whole plastics have never been quantified in birds.

A key question in plastic pollution research includes whether environmentally relevant plastic loads have toxicological impacts on the growth, reproduction and endocrine function in birds, specifically effects on male and female reproductive function and development. We addressed these questions with a three-generation experiment to quantify effects of ingested plastic on growth, reproduction, and endocrine function using a model bird species, the Japanese quail, *Coturnix japonica.* (see Appendix 5: Overview of experimental design).

Methods

<u>Experimental design</u>

We chose Japanese quail, *Coturnix japonica*, as a model species due to its established use as an avian model for endocrine disruption, suitability to captivity and fast growth rate, early onset of maturity, with beginning egg laying at approximately 6 weeks of age [83]. We conducted a laboratory experiment to examine the impact of ingested plastic and test the potential for exposure to substances for endocrine disruption in birds, following the U.S. Environmental Protection Agency's Office of Chemical Safety and Pollution Prevention (OSCPP) test guidelines (OCSPP 890.2100) [83]. Care and use of the animals for this experiment followed the Australian Code for the Care and Use of Animals for the Scientific Purposes - 8th Edition 2013 and was approved by the University of Tasmania Animal Ethics Committee on 02 May 2016.

Japanese quail were sourced as fertile eggs from Rannoch Quail, Nubeena, TAS. Eggs were incubated until hatch in a HovaBator Genesis 1588 Advanced Egg Incubator. For F0 generation total of 240 eggs were set and incubated at 100F and 45% humidity for 14 days, after which humidity was increased 70% for hatching. Eggs that were not hatched 24 hours after the first eggs hatched were considered unhatched. Chicks were moved from the incubator into chick housing. Chicks were divided evenly into five groups. One control group (C), one virgin plastic low treatment group (VL), one virgin plastic high treatment group (VH), one ocean-floated plastic low treatment group (OL), and one ocean-floated plastic high treatment group (OH). Chicks were housed in treatment groups in group chick housings until they reached 4 weeks of age, after which they were paired with a healthy opposite-sex mate (sex was determined by feather characteristics) and moved into a pair housing.

Quail were housed in custom-made housings devoid of plastic to prevent accidental exposure. Cages were constructed from plywood and aviary mesh wire, with Eucalyptus sawdust bedding. Chick housings had 1200x600mm floor area with a 275W heat lamp externally mounted at one end per treatment. Adult pairs were housed in units with each pair having 300x600mm of solid floor space with Eucalyptus sawdust bedding. All bowls for food and water were stainless steel. Bowls for chicks were placed on the floor inside the housing and adult bowls were mounted externally. Food and water was provided ad libitum, water was changed daily, and sawdust bedding was changed weekly. Their diet was Laucke brand "Game Bird Starter" until the birds were 4 weeks of age, after which adults were fed Laucke brand "Game Bird Maintenance".

Plastic prepared to be fed to the quail were polypropylene (PP) production pellets (often called 'nurdles') which are an irregular round shape of 3-4.5ml in diameter and weighing 25-30mg. Virgin plastic treatment birds were fed unaltered "virgin" pre-production plastic pellets. Ocean-floated plastic treatment birds were fed pellets which had been floated at the surface of the Derwent Estuary, Hobart, Tasmania in a mesh bags tied to a post for 6 months to allow photo-degradation, mechanical degradation and absorption of background oceanic pollutants prior to experiment.

Low plastic treatment groups were fed 5 plastic pellets and high treatment groups were fed 10 plastic pellets, the low treatment quantity was based off the mean and high treatment 2x mean of plastic items ingested by a short-tailed shearwater (Chapter 1). F0 generation adult quail were fed two pieces of plastic at a time, by placing pellet at the back of tongue, every second day from age Week 6. F1 generation quail, bred from the parental F0 generation of the same treatment, were treated by giving one plastic pellet every second day from 48 hours after hatch. The F2 generation did not receive a treated diet. Birds allocated to different treatments were housed in the same animal storage facility but otherwise did not come into direct contact nor interact with each other.

Quails were sacrificed at 12-13 weeks of age by cervical dislocation following Australian Code for the Care and Use of Animals for the Scientific Purposes - 8th Edition 2013 guidelines, and members of each generation were culled within a week. Trunk blood was collected by syringe and centrifuged into 2.5ml microcentrifuge tubes to collect serum for hormone analysis. Immediately after death the bird was dissected and liver, spleen, thyroid glands, kidney, cloaca, and male or female reproductive tract were excised and transferred to 10% neutral buffered formalin according to OCSPP 890.2100. All organs were transferred into formalin within an hour of death.

<u>Histology</u>

Formalin fixed tissue was processed routinely for wax or paraffin embedding and sectioning at 5 microns following OCSPP 890.2100. Sections were stained with hematoxylin and eosin for examination by light microscopy. Histology slides were prepared following OCSPP

890.2100. Histology lesions for each organ were scored from 0 (absent) to 4 (severe) using subjective criteria specific to the organ and nature of the lesion, examining all available fields. Histology was first scored in the control, ocean-floated high and virgin high treatments of the F1 generation where the greatest effect was expected to be found. Where treatment associated lesions were found, we scored that organ for all birds. Where no treatment associated lesions were found in F1 control or high treatment groups, we discontinued histological assessment of this organ.

Hormone analysis

Sample extraction

Testosterone (T) and 17 β estradiol (E2) were extracted from plasma following OCSPP 890.2100 by modification of the protocol of Parsley et al. 2014 [84]. One milliliter of plasma was extracted with 2.0 ml of AR grade diethyl ether (Merck Millipore, Australia), and snap frozen in a bath of dry ice-cooled methanol (Sigma Aldrich, Australia). The diethyl ether was then transferred to a clean test tube and the extraction process was repeated three times. The diethyl ether was then evaporated to dryness under a stream of nitrogen. The samples were immediately resuspended in AR grade absolute ethanol (Merck Millipore, Australia). The extraction efficiency was 91 and 96 % for T and E2 respectively.

Radioimmunoassay

Duplicate 500 µl aliquots of plasma extracts were incubated overnight with 2,4,6,7 3H-estradiol or 1267 3H testosterone (PerkinElmer, Australia) and testosterone or estradiol antiserum (Novus Biologicals, testosterone 14PC28, estradiol NBP1-78621, In Vitro Technologies, Australia) in phosphate buffered saline-gelatin. The standard curve ranged from 3.125-800 pg of authentic T and E2. The sensitivity was 1.5 pg, assay parallelism was demonstrated by varying the amount of extract and obtaining equivalent results. The inter assay and intra assay coefficients of variation was 11.4 and 12.2 % respectively. All results were corrected for extraction efficiency.

Statistical analysis

Statistical analyses were performed using statistical software R (Version 3.3.3) (32). We used one-way ANOVAs to test for the differences in continuous outcomes between each treatment group and for each sex individually, including body weight each week, total number of eggs laid, and histology lesions scored 0-4. Where significant results were found, we used post-hoc pairwise t-tests with Bonferroni corrections to examine differences between individual groups. Generalized linear models (GLMs) were used for discrete binomial outcomes including survival and sex ratios.

Results

Contrary to expectations, we found most variables, including mortality, adult body weight, and hatching to 14-day body weight were not significantly different between treatments; or the treatment effects did not exceed normal limits [83] (Appendix 1, Appendix 5). We also found no significant difference between treatments in the number of eggs laid, frequency of abnormal eggs, egg fertility, embryo weight, yolk weight, and the eggshell break force of eggs. We found no significant difference in circulating testosterone and estradiol concentrations, nor treatment-related lesions or histological changes in morphology of the liver, spleen, thyroid glands, kidneys, cloacal glands and female reproductive tract (ovary, infundibulum, magnum, isthmus, uterus and vagina) in high-dose plastic exposure first generation (F1) birds. As no effect was found in these high-dose birds, less exposed F1 low dose, parent generation (F0) and second generation (F2) birds' organs were not assessed.

Three clear effects of plastic ingestion were observed: slower growth in chicks (Figure 1), and subsequent delayed sexual maturity in females (Figure 2) and an increased frequency of cysts in the reproductive tract of adult males (Figure 3, Figures 4a-d) . In the male reproductive tract, the presence of epididymal epithelial cysts (EECs) were more frequent and of greater severity in F0 and F1 plastic fed birds (Figure 3, Figures 4a-d) than in controls (F0, $R2= 0.0628$, $P=0.039$, F1, $R2= 0.101$, $P=0.043$). Ocean-floated plastic fed and virgin-plastic fed treatments did not differ significantly from each other. There was a significant difference in in the weights of 3 to 6-week old chicks of the F1 generation among treatments ($P<0.05$), with control chicks being the heaviest (Figure 1). There was also a difference in time to sexual maturity in females in F1 ($P=0.014$), with controls "C" reaching sexual maturity before treatment groups fed plastic (Figure 2).

Discussion

Despite the ubiquity of plastic in many avian species, we did not find evidence of endocrine disruption resulting from experimental plastic ingestion across most of the measures examined in Japanese quail. This may be positive news for plastic ingesting birds in general, as it suggests that the toxicological health implications of plastic ingestion are not as serious as commonly assumed, and reflects similar experimental findings of an absence of imminent harm to fish experimentally fed plastic [85].

Perhaps the most interesting finding was the presence of epididymal intra-epithelial cysts (EECs) in plastic fed males (Figure 3). EECs are rare in Japanese quail [83], and have not been noted in other bird species, although they have been observed in reptiles [86], amphibians and mammals [87-90]. EECs occur in mice experimentally injected with estrogens, where they are believed to be an estrogenic developmental effect [88]. The presence of EECs in birds fed plastic, scaling with both the quantity of plastic fed, as well as the type across both F0 and F1 generations, demonstrates these EECs are likely to be an endocrine response to plastic ingestion. Nonetheless, this increase in EECs did not affect the fertility of eggs laid by Japanese quail pairs. Many plastic-ingesting bird species, however, especially seabirds, breed over many decades [7, 16] highlighting the need for further research on chronic plastic exposure; in particular, we need to know about the presence and long-term changes of EECs and their effects on male reproductive health and success on long term breeding (k-selected) species.

The second major finding of slower growth in juvenile birds fed plastic (Figure 1) mirrors the results of Ryan [25] in male domestic chicks fed plastic pellets. We hypothesize that this is primarily a physical effect, due to dietary dilution caused by plastic occupying gut volume, which results in reduced ingestion of food. It is possible that there was also a toxicological effect because the growth rate of virgin plastic fed high-treatment birds was greater than ocean-floated plastic fed high-treatment birds (Figure 1), as ocean-floating plastics are chemically and physically altered by weathering [80], but the mechanism of any toxicological effects are unclear. The 1.29-5.95 day delay in the onset of sexual maturity of plastic-fed females (Figure 2) possibly follows their reduced growth as chicks, as has been observed in domestic chicken hens following feed access restriction [91, 92]. As the delay in sexual maturity was more pronounced in ocean-floated plastic fed to birds, we propose that there is again also a potential chemical effect, although we do not know the mechanism by which this occurs. Importantly, the birds who initially experienced reduced growth showed no size difference by adulthood at week 7 and showed the same reproductive output as other treatments. Any initial differences we found do not appear to result in on-going disadvantage for individuals, at least in this rapidly maturing and highly fecund species [83].

Though toxicological studies of plastic effects on wild birds are few, several have sought to explore toxicological effects of plastic ingestion in seabirds. Among these studies, concentrations of sorbed chemicals including PCBs [31] and metals [93] scaling with ingested plastic loads are detected [31], with consequent health effects including reduced body weight found in chicks [93], but not adults [31, 94], reflecting our findings in quail. Other studies found no link between ingested plastic and sorbed chemicals or toxicity [95, 96]. As with quail, authors of seabird studies finding reduced chick growth associated with plastic ingestion suggest this likely results from reduced stomach capacity [93], rather than being a toxicological effect. In this experiment we tested only polypropylene plastic, and it is possible that toxicity may differ were different plastic types examined. The paucity of evidence of toxicological effects despite increasing attention to plastic ingestion further supports that among seabirds, like quail, plastic ingestion toxicity may not be a major source of harm, though further study is needed.

We found few effects of plastic ingestion across many variables assessed in this highly fecund, short-lived, precocial, indeterminate laying species. We observed short-term growth and maturity delays that resolved at adulthood. However, we acknowledge that the laboratory conditions under which this experiment took place may not represent survival pressures facing wild birds, particularly taxa with dissimilar life histories under natural conditions. For instance, a reduction in chick weight in wild birds and consequent late fledging reduces post-fledging survival in some species [29, 30, 97, 98]. Many plastic ingesting species, including seabirds [7, 12] and shorebirds [22, 99], have highly migratory and long-lived life histories. If the delayed growth and maturity detected in Japanese quail similarly affect these species, fledglings would be vulnerable to increased mortality resulting from reduced fledging body weight or late fledging as a consequence of their migratory lifestyle.

In conclusion, the toxicological effects of plastic ingestion do not appear to be a significant impediment to Japanese quail health and survival and would likely not cause population-level effects in similar species. The physical aspects of plastic ingestion affected growth rate due to reduced stomach volume and energy intake, although this did not affect survival or population outcomes for Japanese quail over the course of several generations. Although toxicological and endocrine effects seem not as severe as feared for the millions of birds and other wildlife carrying small, sub-lethal plastic loads, the group which most frequently ingest plastic, the seabirds, exhibit life histories most vulnerable to the consequences of plastic ingestion.

Figures

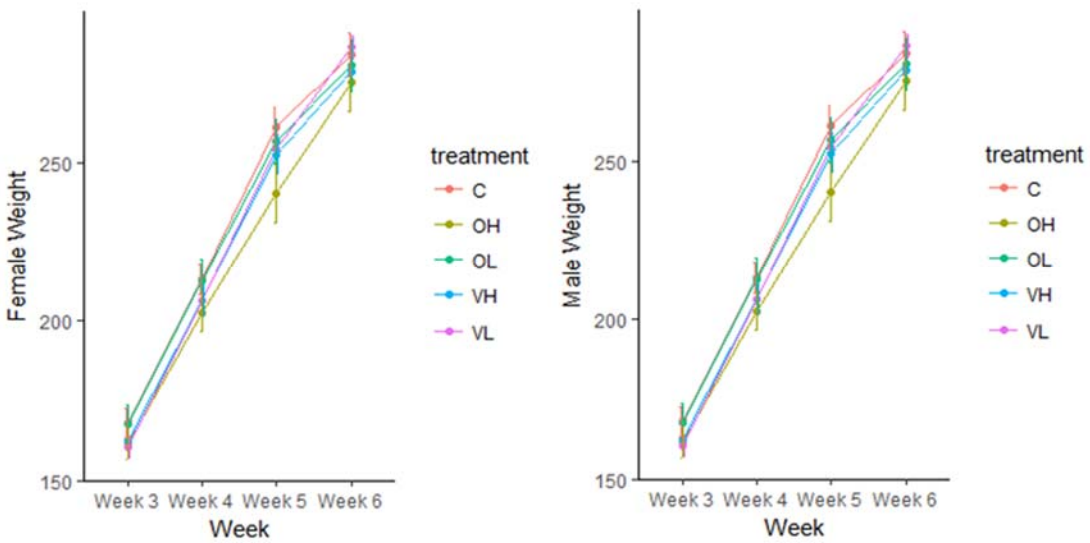


Figure 1. Body weight of Japanese quail F1 generation chicks aged 3 and 6 weeks. This figure compares five treatment groups: control group (not fed plastic) (C), virgin plastic low dose treatment (VL), virgin plastic high dose treatment (VH), ocean-floated plastic low dose treatment (OL) and ocean-floated plastic high dose treatment (OH). There was a significant difference in chick body weight between treatment groups for weeks 3-5.

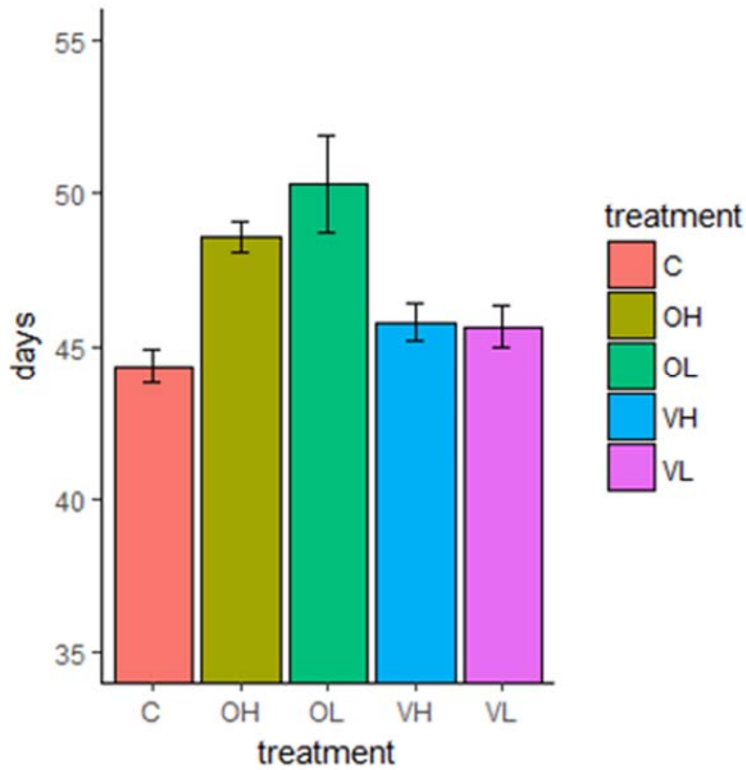


Figure 2. Age of Japanese quail hen when the first egg was laid in the F1 generation, representing the onset of female sexual maturity. This figure compares five treatment groups: control group (not fed plastic) (C), virgin plastic low dose treatment (VL), virgin plastic high dose treatment (VH), ocean-floated plastic low dose treatment (OL) and ocean-floated plastic high dose treatment (OH).

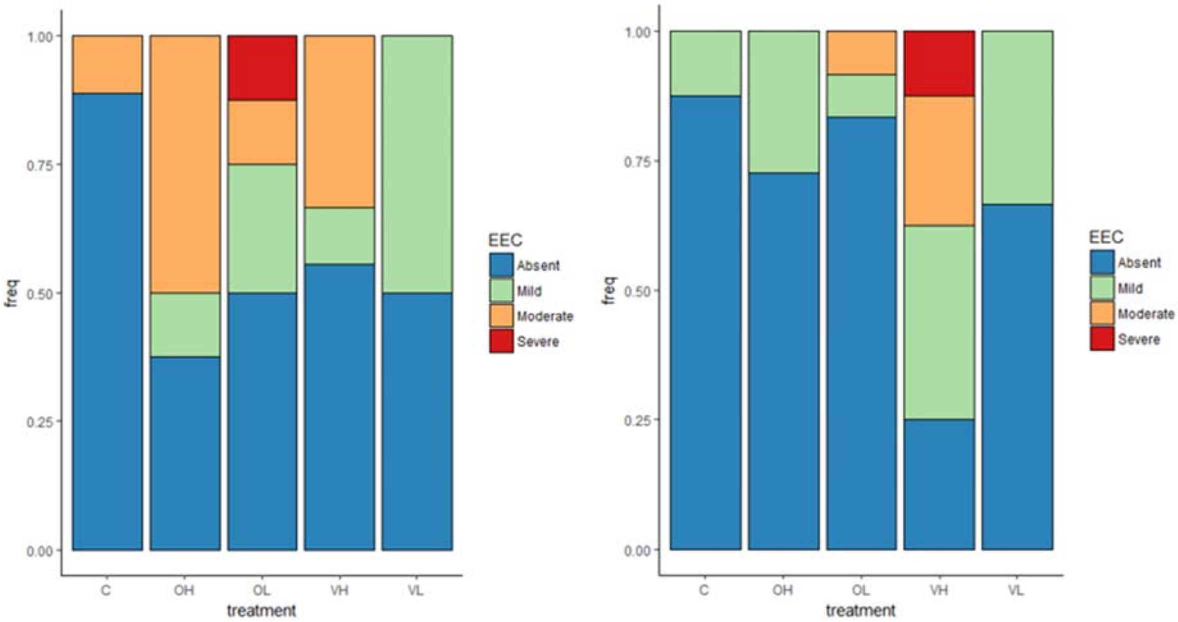
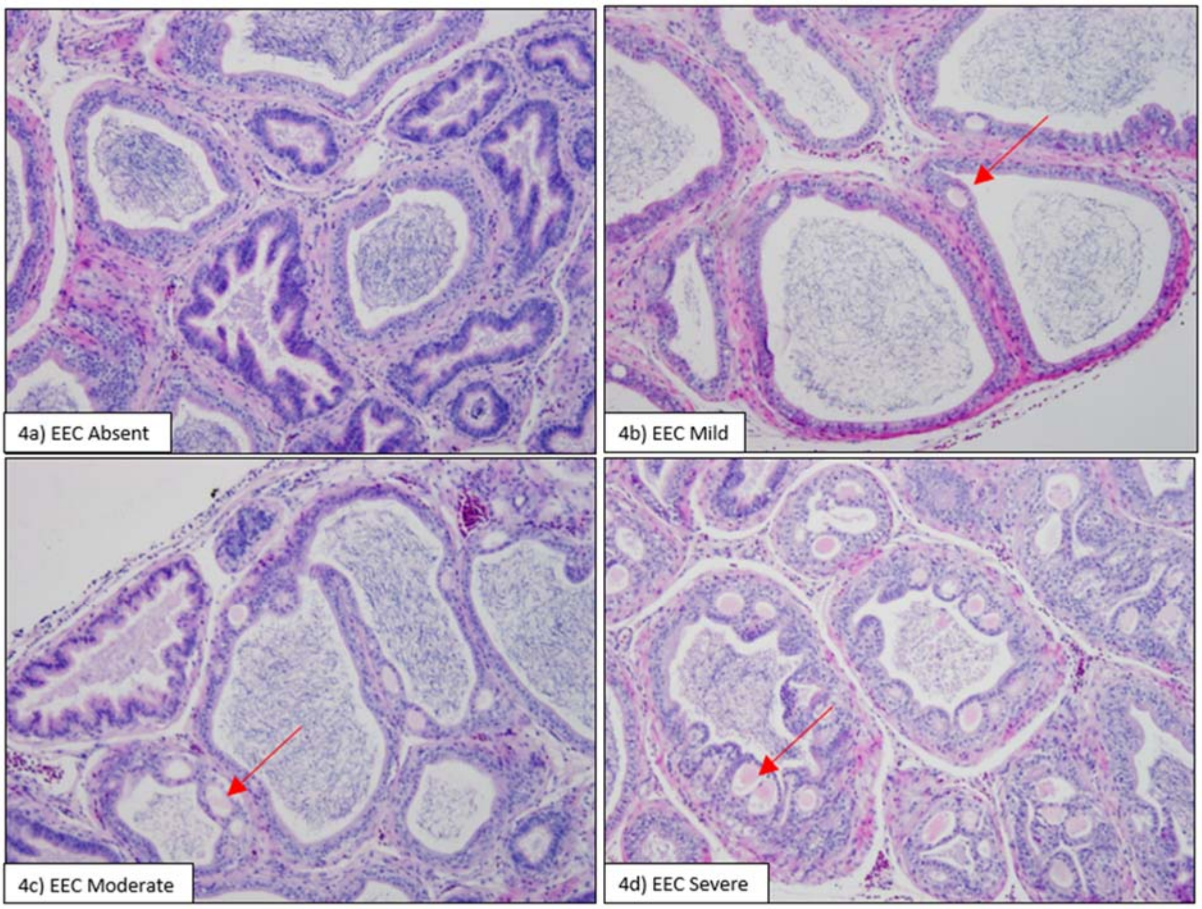


Figure 3. Presence and severity of epididymal epithelial cysts (EECs) found in the reproductive tracts of male Japanese quails in F0 (left) and F1 (right) generations. This figure compares five treatment groups: control group (not fed plastic) (C), virgin plastic low dose treatment (VL), virgin plastic high dose treatment (VH), ocean-floated plastic low dose treatment (OL) and ocean-floated plastic high dose treatment (OH).



Figures 4a-4d. Epididymis of 12-week old adult male Japanese quail. Figs 4a through 4d represent progressing grades in severity of epididymal intra-epithelial cysts (EECs). Figure 4a shows the absence of EECs in a control male, figure 4b shows mild EECs in a single control male. Figure 4c shows moderate EECs in a virgin plastic high treatment male, and figure 4d shows severe EECs in a virgin plastic high treatment male. These images were taken at 10x magnification.

Supplementary Materials

Appendix 1: Table of Japanese quail reproduction results that were not statistically significant

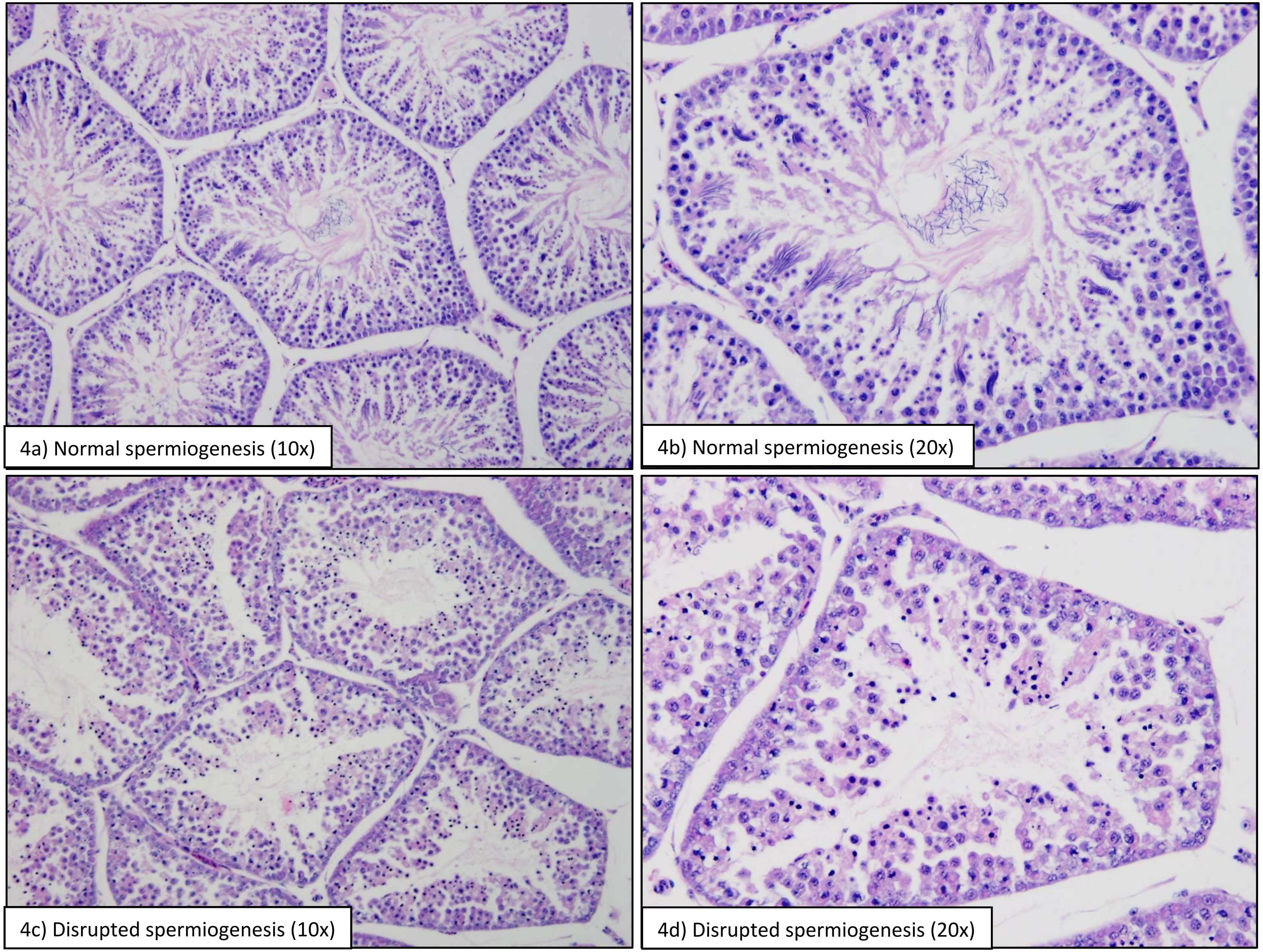
	% eggs set to hatch that hatched	Ratio males : females	% mortality within first 14 days	% adult mortality (excluding accidental death)	Mean # weekly eggs between weeks 6-12	Mean # abnormal eggs per week	Eggshell break force (N)	Mean eggshell thickness (mm)	% Eggs set for embryos that were infertile	% fertile eggs set for embryos alive at ED15	Offspring day 15 embryo + yolk weight (g)	Offspring day 15 embryo weight (g)
F0 generation (240 eggs set to hatch)												
C	77.9%	1:0.86	4.8%	6.25%	5.38±0.11	0.018±0.01	10.1±0.6	0.19±0.005	8.3%	59.1%	8.4±0.2	6.1±0.1
VL				0%	5.35±0.27	0.053 ±0.02	9.2±0.6	0.18±0.005	0%	66.7%	8.6±0.3	5.8±0.2
VH				0%	5±0.2	0.018± 0.01	9.5±0.6	0.17±0.006	0%	81.8%	9.3±0.1	6.5±0.1
OL				9%	5.48±0.1	0.126± 0.04	9.6±0.6	0.18±0.003	15%	70.6%	8.4±0.3	6±0.2
OH				0%	5.37±0.14	0.018± 0.01	8.3±0.5	0.2±0.006	10%	83.3%	9.1±0.2	6.3±0.2
F1 generation (per generation 48 eggs set to hatch, 24 eggs set for embryos)												
C	68.75%	1:1.38	6%	6%	3.98±0.22	0.09± 0.02	10.1±0.5	0.22±0.006	4.2%	87%	7.7±0.2	5.4±0.2
VL	75%	1:1.12	0%	0%	4.12±0.19	0.26± 0.04	10.5±0.3	0.21±0.005	4.2%	87%	8.1±0.2	5.6±0.2
VH	68.75%	1:0.88	3.3%	0%	4.32±0.12	0.13± 0.04	9.9±0.4	0.2±0.006	0%	82.6%	7.9±0.2	5.5±0.2
OL	66.67%	1:0.61	6.25%	6.25%	4.17±0.18	0.1± 0.04	9.9±0.5	0.2±0.007	12.5%	81%	7.8±0.3	5.5±0.2
OH	64.58%	1:0.94	6%	0%	3.98±0.18	0.18± 0.05	10.1±0.5	0.22±0.004	8.7%	81%	8.2±0.3	4.6±1.1
F2 generation (per generation 24 eggs set to hatch, 24 eggs set for embryos)												
C	41.67%	1:1.33	0%									
VL	87.5%	1:0.9	9.5%									
VH	62.5%	1:0.58	0%									
OL	50%	1:2	0%									
OH	58.3%	1:1	14.3%									

Appendix 2: Table of mean male Japanese quail body weight (g) by treatment group and standard error

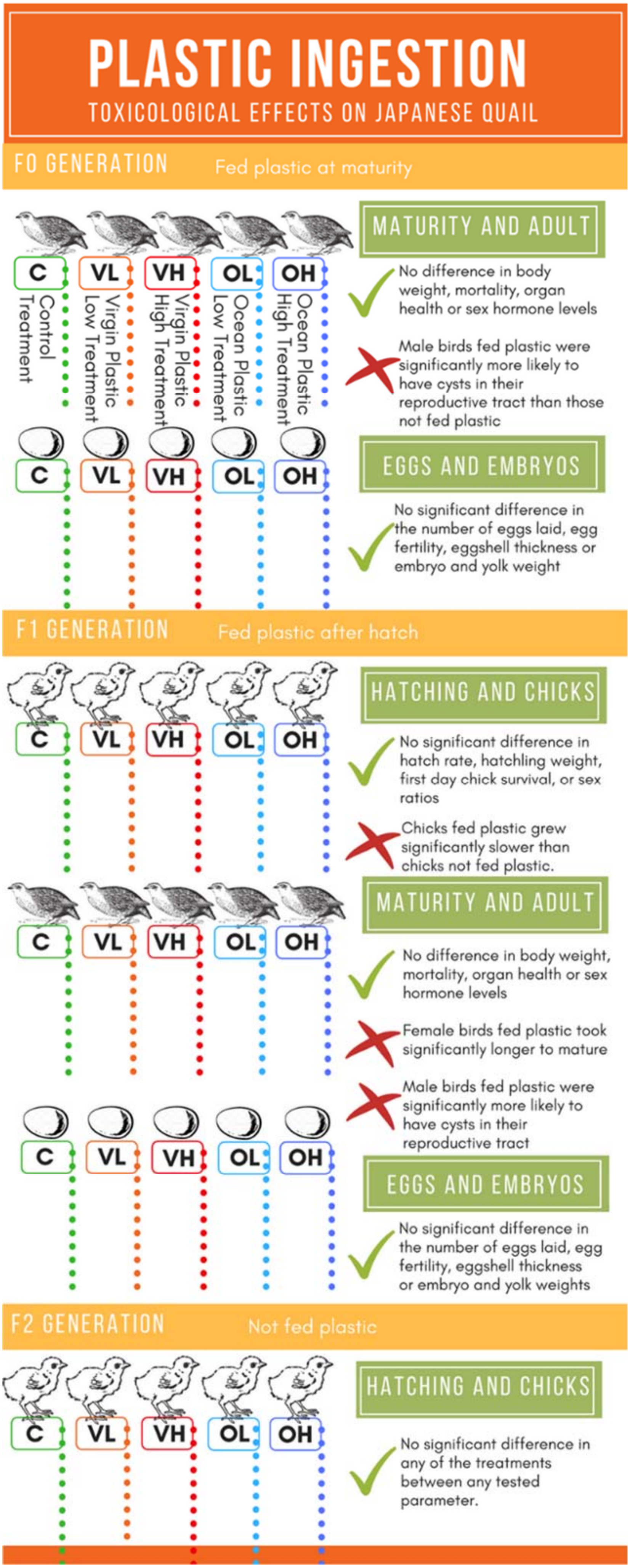
Male	Hatch	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
F0 generation													
C							247.5±6.2	249±7.3	254.2±5.8	260.2±6.6	263±6.5	261.7±8.4	266.6±7.3
VL							247.8±5.4	251.8±6	254.6±6.7	260±7	264±6.2	264.7±7	268.8±6
VH							241.3±5.4	247±6.3	250.4±6.2	255.4±6.4	258.7±6.2	264.2±5.9	262.6±6.9
OL							249.8±3.6	255.5±4.9	263.7±5.4	268.2±6.4	275.6±7	276.5±7	264±7.6
OH							256.6±5.8	257.6±5.9	261.9±6.6	265.9±6.9	268.2±7	273.5±6.8	262±6.9
F1 generation													
C	8.7±0.2	23.7±0.7	86.8±2.2	164.8±4.2	200.2±5.4	224.5±4.7	241.5±6.9	240.3±7.4	NA	266.3±5.6	NA	264.6±5.6	NA
VL	8.9±0.2	22.9±0.6	81.7±1.4	160.3±2.4	193.1±3.3	219.7±5.9	237.6±5.8	231.3±5.7	NA	251.9±9.9	NA	255.5±9.7	NA
VH	8.9±0.2	24.4±0.8	87.4±2.3	160.8±3.3	202.9±4.2	217.8±5.2	234.1±4.8	235.1±5.9	NA	253±6.3	NA	251.7±7.5	NA
OL	8.6±0.2	21.9±0.8	85±2.4	161.4±3.9	209.5±3.2	233.9±3.7	243.1±4.1	241.2±5.4	NA	258.6±5	NA	262.2±5.6	NA
OH	9.1±0.2	21.7±0.6	74.7±1.7	147.6±4.9	187.4±5	210.1±7.5	230.8±8.2	226±5.1	NA	245.4±6.1	NA	249.9±6.8	NA
F2 generation													
C	9±0.3	78.7±4.4	135.3±13										
VL	9±0.2	74.8±1.9	149.7±3										
VH	8.8±0.2	73.3±2.6	146.4±4.3										
OL	8.5±0.1	81.3±2.7	141.3±7										
OH	8.6±0.5	76.5±3.6	127.5±9.1										

Appendix 3: Table of mean female Japanese quail body weight (g) by treatment group and standard error

Female	Hatch	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
F0 generation													
C							283.9±6.2	283.2±7.3	291.2±7.8	301.1±6.3	311.1±7.1	303.1±10.4	307.5±9.4
VL							289.7±4.2	279.5±7.5	288.1±6.9	293.2±6.2	304.5±6.5	300.6±5.7	301.6±6.5
VH							291.4±6	286.5±7	292.1±6.5	304.2±6	312.6±8.1	308.6±7.9	262.6±7.9
OL							297.2±4.9	299±6.6	305.2±8.4	309.1±11.4	315.4±9.9	315.5±10.4	292.7±11.7
OH							285±7.3	281.1±8.1	286.9±7.1	302.7±7.3	303.8±6.6	311±7.8	288±9.3
F1 generation													
C	9.1±0.3	23.7±0.7	85.5±2.1	168.2±4.6	213.2±4.9	260.8±6.7	283.8±6.9	248.9±7.9	NA	287.5±9.2	NA	297.2±9.5	NA
VL	8.7±0.2	22.9±0.6	85.1±1.3	160.6±3.5	206.5±3.4	254.5±4.7	285.9±3.6	270.3±5.7	NA	293.6±8.5	NA	301.4±11.1	NA
VH	9.3±0.2	24.4±0.8	87.1±2.6	162.5±3.5	206.6±4.8	252.2±5.7	278.2±6	262.1±6.3	NA	282.2±6.8	NA	282.1±7.5	NA
OL	8.8±0.3	21.9±0.8	89.4±3.2	167.8±5.6	212.5±6.7	256.5±6.8	280.5±8.2	262.3±11.1	NA	273±16.2	NA	299±9.8	NA
OH	9±0.2	21.8±0.6	77.8±3	161.5±5	202.8±5.9	240.3±9	275.1±9.2	273.8±6.6	NA	290.9±7.3	NA	295.4±9.8	NA
F2 generation													
C	8.2±0.3	76±6	146.25±3.6										
VL	8.9±0.3	74.8±1.9	151.4±2										
VH	8.7±0.4	73.3±2.6	143.1±4.1										
OL	8.5±0.3	81.3±2.7	160.5±2.9										
OH	8.9±0.4	76.5±3.6	147.5±2.1										



Appendix 4a-d. Testes of 12-week old adult male Japanese quail. Appendix 4a and 4b show normal spermiogenesis in a control male at 10x and 20x magnification respectively. Appendix 4c and 4d show disrupted spermiogenesis found in one single F1 ocean plastic high treatment male at 10x and 20x magnification respectively. It is not known whether this disrupted spermiogenesis is treatment related.



Appendix 5. Overview of experimental design and findings

Discussion Chapter: Estimating the global mortality of Procellariiform seabirds resulting from plastic ingestion

Lauren Roman[1], Britta Denise Hardesty[2], Mark A. Hindell[1,3], Chris Wilcox[2]

1 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia,

2 CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia,

3. Antarctic Climate and Ecosystems CRC, University of Tasmania, Hobart, Tasmania, Australia,

Summary

Oceanic plastic pollution is a global issue and is known to affect seabirds that commonly ingest marine debris, causing death when indigestible items become lodged in the digestive tract. Estimates suggest 99% of all seabird species will have ingested plastic by 2050. Debris ingestion is a particular concern for Procellariiform seabirds, of which 44% of species are globally threatened, while over 50% are declining. Despite the prevalence of plastic ingestion, its contribution to global seabird populations is unknown. Here we integrate three models; global seabird debris ingestion incidence, seabird debris incidence-load conversion and seabird ingested debris load mortality, to estimate the average global mortality for individual seabirds across all Procellariiform taxa. Individuals in over 60% of species have a ≥5% chance of dying from ingesting debris while individuals of the top nine species have a ≥30% chance of debris-related death. Shearwaters, fulmarine petrels and prions are the groups most likely to die from debris ingestion, and they would benefit most from initiatives to reduce the amount of debris entering the marine environment. This is the first examination of the contribution marine debris poses to seabird mortality and it quantifies the species-level threat with a robust analytical approach.

General discussion

There are 139 Procellariiform species, of which 52.5% are declining and 44% are globally threatened [17]. Seabirds are declining faster than any other group of birds, through a combination of the threat from commercial fisheries, pollution, invasive predators, habitat degradation and human disturbance [67]. Although the contribution of plastic pollution to their decline has been the subject of much conjecture, it remains unquantified and untested. Understanding the contribution of plastic ingestion to mortality and the resulting population consequences is considered a priority for assessing the importance of mitigating the plastic pollution effect on wildlife globally [50].

Plastic pollution kills seabirds primarily because indigestible items become lodged in the gastro-intestinal tract (Chapter 3). The effect of additive and sorbed plastic-associated chemicals on seabird survival has not been quantified, but recent studies suggest that mortality from toxicological effects is likely to be negligible [77, 95] (Chapter 4). Although a seabird can die from ingesting a single debris item, the risk of death increases as the bird is exposed to and ingests an increasing load of debris (Chapter 4). The extent of plastic pollution a seabird is exposed to is an important factor determining how much plastic an individual is likely to ingest (Chapter 2). Seabirds whose foraging range overlaps with more heavily polluted regions ingest higher loads of plastic pollution than those in less polluted environments (Chapter 2).

We predicted the expected seabird mortality by combining the exposure of Procellariiform species to debris in their environment, modelled in previous studies [1], with the dose-response relationship between debris ingestion and debris-related cause of death (Chapter 3) (Figure 2, see Supplementary information: Methodology). We used a general additive model (GAM) to determine the relationship between the incidence of debris ingestion and expected mean load of ingested items by a species using known debris incidence and ingestion data from 51 species (Chapter 2), weighted by the number of individuals examined in each species (Figure 1). Mean debris loads for each species, generated by this model, were then compared to the dose-response mortality curve for each species. This predicts the expected mortality for each data-supported species, excluding those species where debris ingestion data was not available (Supplementary information: Methods; Table 1).

Ingestion of debris is a major source of mortality for seabirds; of the 62 species with sufficient data, individuals in 31 species had a probability of death due to plastic of less than 10%, one species had a probability of individual mortality between 10 and 20%, 21 species a probability of 20-30%, and nine species a probability of 30-40% (Figure 3).

Debris ingestion is a more serious threat for some Procellariiform groups than others. Shearwaters, fulmarine petrels and prions are most represented among species with a high probability of debris-related cause of death (Table 1). Although storm-petrels were not assessed due to lack of incidence data [1], they have a high incidence of plastic ingestion comparable to or exceeding that of prions [64] (Chapter 2) and are likely to have high debris-related mortality. Albatrosses and gadfly petrels are least likely to die from debris ingestion. For albatross, this is presumably due to their lack of a restricted isthmus juncture in their gastro-intestinal tract, the site where debris obstructions generally occur (Chapter 3), that prevents regurgitation of indigestible items. It is not known why some gadfly petrels have low mortalities, but we speculate it may be due to many gadfly petrel species foraging individually rather than in flocks or rafts [16] where hasty intraspecific competition may drive birds to make less selective foraging decisions. Species most likely to die from debris ingestion would benefit most from initiatives to reduce the amount of debris entering the marine environment.

Expert elicitation has identified debris ingestion as a likely severe threat to marine fauna [77], however, debris ingestion is absent from lists of global assessments of threats to seabirds [67]. Debris is listed as a potential threat for only ten species by the International Union for Conservation of Nature [17] and no plastic pollution conservation actions are recommended [17]. This perceived lack of a threat is possibly due to a paucity of mortality data and a lack of visibility of debris-related deaths, compared to more visible mortality from fisheries bycatch and nest predation [67]. With the high predicted debris-related mortality from our analysis, two pressing questions arise: 1) where are all these dead birds? and 2) why aren't more populations declining?

Regarding the first question, Procellariiform seabirds are pelagic by nature, and most of their deaths occur unobserved at sea, with mortality occurring at rates consistent with seabird density [100]. Carcasses that are found are usually individuals killed in fisheries, following oil spills, or as starving seabirds pushed ashore following storms and adverse weather in wreck events [101] and are not representative of the wider population. The most representative deaths would be beach-washed individuals not associated with wrecks, and even these account only for the birds that die within a small, unknown radius of the coastline. Even if large numbers of seabirds are dying from ingesting debris, the chance that the carcass would be encountered, and the cause of death discovered would be very low.

Regarding the second question, our study documents seabirds' mortality and ingested debris load observed over an unknown period of its lifetime. With the exception of juveniles, it is unknown whether the adult birds in the study averaged four or forty. We also do not know the turnover rate for the ingested debris loads and assume the debris load at death is representative of the average load that lead to the death for the bird, and that individuals in the study represent an average for the species. Sampling seabirds at random, during a random point during their life, shows that healthy wild seabirds carry ingested plastic loads [54], apparently causing negligible harm and not reducing digestion efficiency (Chapter 3) [24, 102]. Estimates of residency time of a debris item in a seabird vary from as little as a month [103] to more than two years [24]. Each "turnover" of ingested debris presents a new opportunity for mortality if the next item ingested, by the nature of its shape or material, may create an obstruction resulting in death. As the duration of a bird's lifetime and number of ingested items increases, so too does the risk that a deadly item is ingested. For this reason, the longer a bird lives, and the more debris it ingests, the higher its chance of mortality due to becoming unlucky by selecting a deadly item during debris ingestion. We expect that debris mortalities in general are skewed towards juvenile birds or older birds. The skew towards juvenile birds results from less selective, naïve foraging, especially among shearwaters and albatross [104] while the skew towards older birds is due to an accumulation of opportunities to ingest debris over their long lifespan. There may not be dramatic population-level effects, particularly in abundant species, if deaths are skewed towards first year birds with naturally high mortality [105, 106] who may not have reproduced anyway, and older birds who have already reproduced for many years. If there is a reduction in seabirds as a consequence of debris ingestion, it's possible that surviving birds may benefit from increased fitness from a type II function response of less prey competition, which would also consequently mask population decline.

Plastic ingestion is a significant cause of mortality across many Procellariiform species. Regardless of the uncertainty surrounding the relative contribution of debris ingestion mortality to seabird population decline, over half of species are indeed declining [17]. Management authorities considering conservation actions should consider this threat to global seabird population declines. Estimated annual mortality from plastic ingestion is the next step in quantifying the scale of this threat. This study increases our quantitative understanding that plastic pollution is a major contributor to the mortality of threatened marine fauna.

Figures

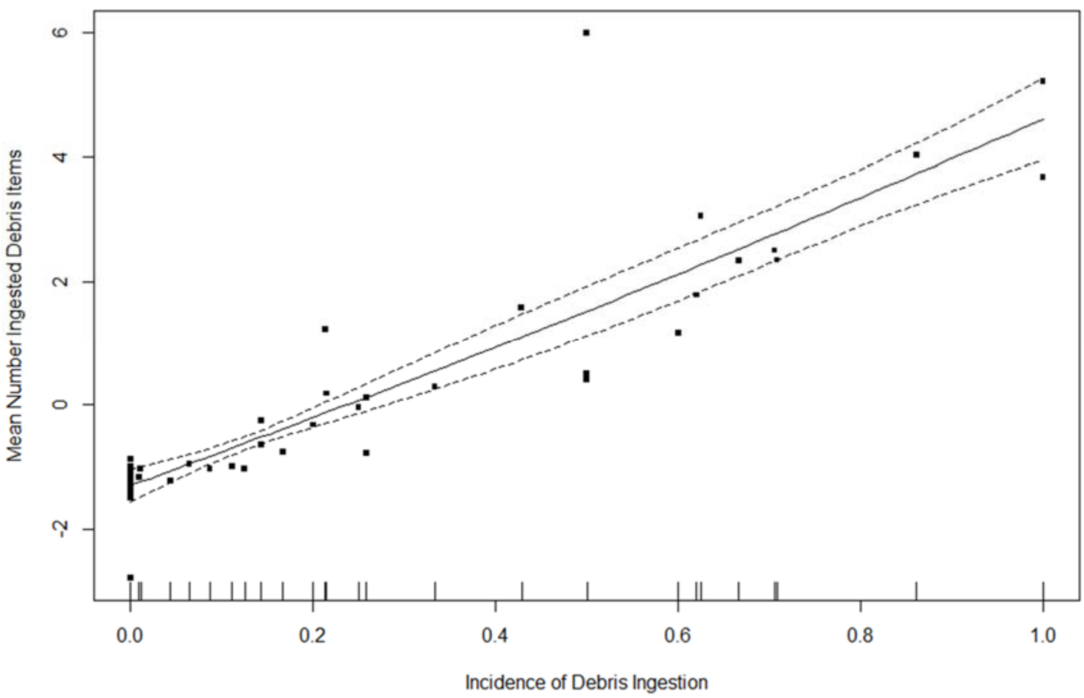


Figure 1. Conversion general additive model (GAM) comparing the mean number of debris items ingested by a Procellariiform species and smoothed incidence of marine debris ingestion (% of individuals with marine debris) in that species, factoring also bird group and species average weight (Chapter 1). Each data point represents a Procellariiform species, and each point is weighted by the number of individuals surveyed in Chapter 1. ($P<0.01$, $R^2 = 0.902$, deviance explained = 92.2%)

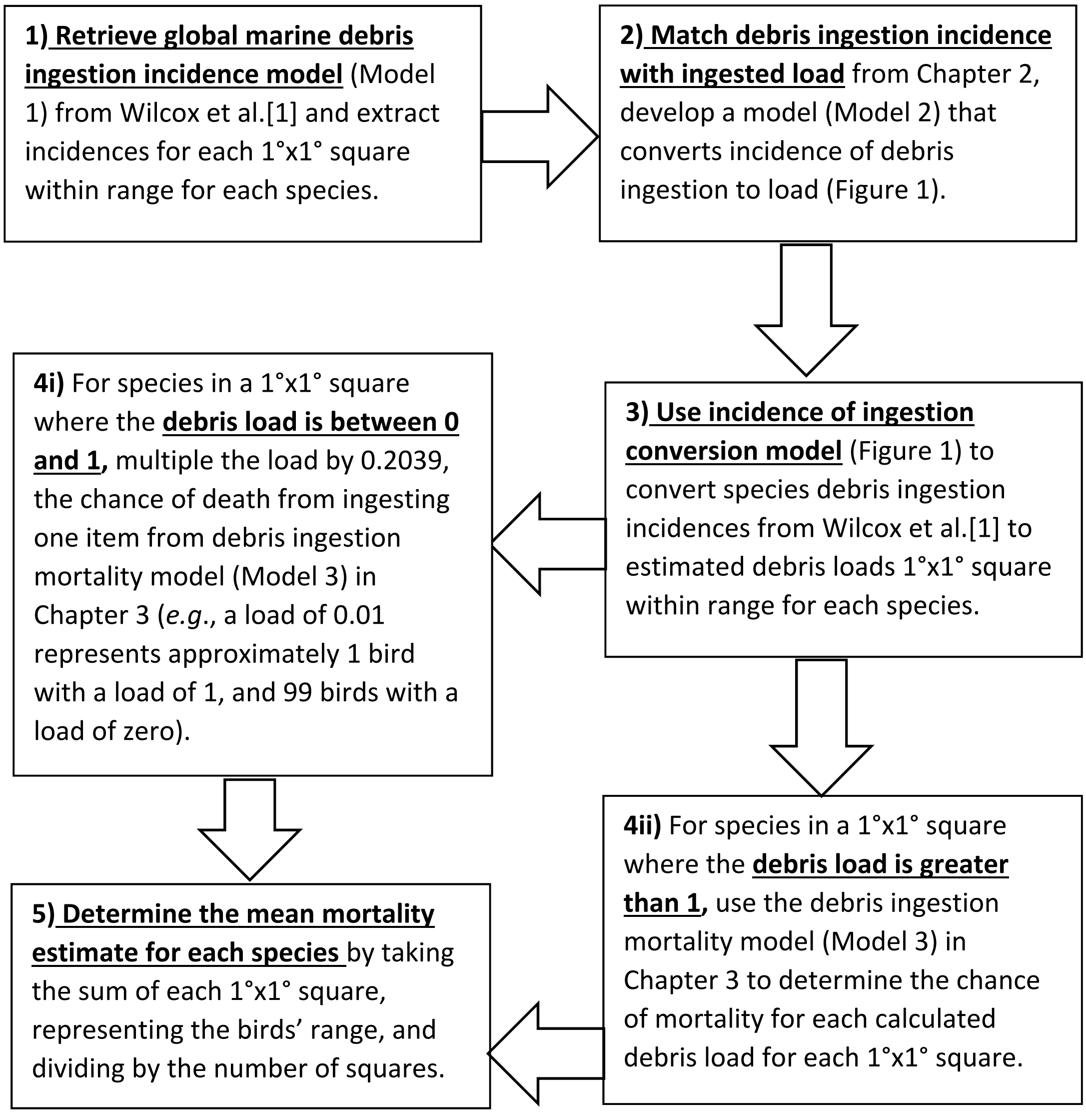


Figure 2. Flow chart demonstrating the methodology which integrates three models to estimate the average global seabird mortality across oceans and Procellariiform taxonomic groups globally; global seabird debris ingestion incidence (Model 1), seabird debris incidence-load conversion (Model 2) and seabird ingested debris load mortality model (Model 3). Detailed methodology is available in Supplementary Information.

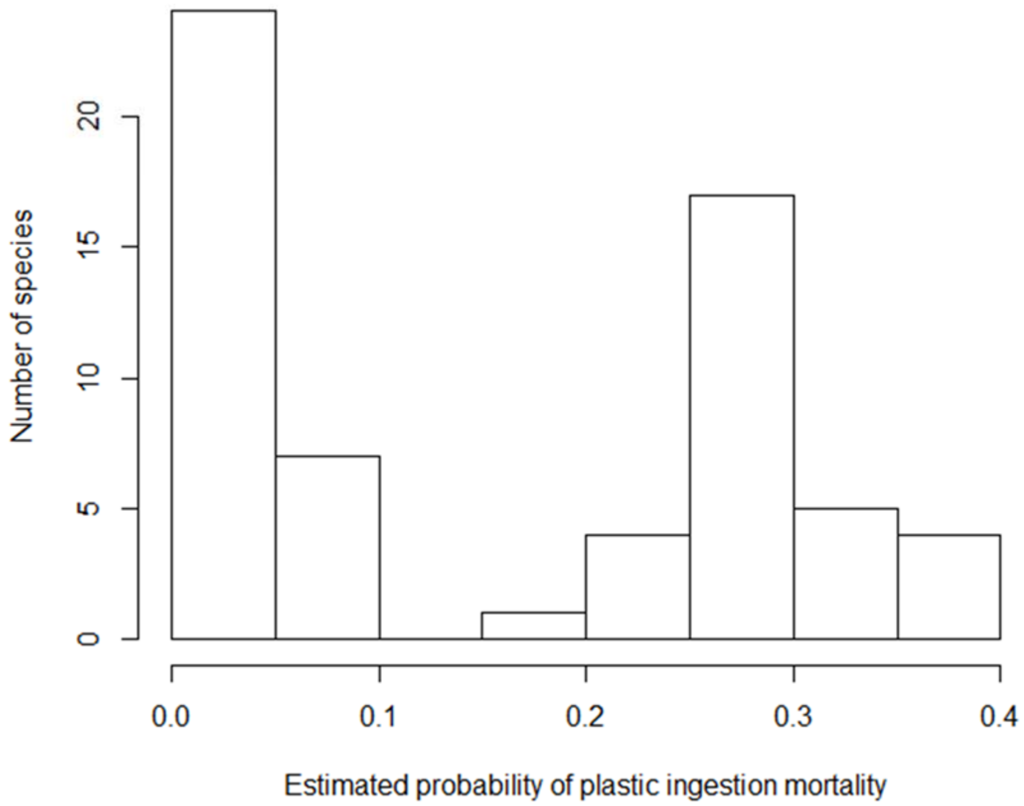


Figure 3. Histogram showing frequencies of the estimated probability of an individual species member dying from a debris ingestion related cause.

Table 1. Estimated mean and standard error of probability of death by plastic ingestion related cause for each species. These values were obtained by multiplying the mean debris load ingested by each species by the dose-response relationship between ingested debris load and debris-related cause of death from Chapter 4.

Bird Group	Species	Mean ingested debris incidence	Std error debris incidence	Mean expected debris load	Std error debris load	**Predicted mortality**	Std error predicted mortality
Albatrosses	Amsterdam Albatross	1.05E-13	1.38E-18	0.157024	9.48E-18	**0.032026**	1.94E-18
	Atlantic Yellow-nosed Albatross	0.073535	6.83E-07	0.219293	3.75E-06	**0.044726**	7.66E-07
	Northern Royal Albatross	7.27E-09	1.15E-15	0.169201	6.33E-15	**0.03451**	1.29E-15
	Sooty Albatross	4.58E-08	1.24E-13	0	0	**0**	0
	Wandering Albatross	5.59E-08	5.61E-14	0.359961	3.08E-13	**0.073417**	6.27E-14
	Whitecapped Albatross	0.889848	1.28E-07	5.162747	8.01E-07	**0.383592**	3.22E-08
Fulmarine petrels	Northern Fulmar	0.875662	1.12E-07	5.009086	6.99E-07	**0.377415**	2.81E-08
	Southern Fulmar	0.908205	6.29E-08	5.221297	3.95E-07	**0.385946**	1.59E-08
Gadfly petrels	Atlantic Petrel	0.229057	1.37E-06	0.377803	7.67E-06	**0.077056**	1.56E-06
	Barau’s Petrel	0.164976	3.93E-07	0.011408	2.18E-06	**0.002327**	4.45E-07
	Bermuda Petrel	0.117853	5.60E-06	0	0	**0**	0

Black-capped Petrel	0.219595	2.19E-06	0.305464	1.22E-05	**0.062302**	2.49E-06
Black-winged Petrel	0.097966	8.58E-08	0	0	**0**	0
Bonin Petrel	0.110802	3.86E-07	0	0	**0**	0
Chatham Petrel	0.110653	9.90E-08	0	0	**0**	0
Collared Petrel	0.106701	1.84E-08	0	0	**0**	0
Cooks Petrel	0.106709	2.78E-07	0	0	**0**	0
Galapagos Petrel	0.180493	3.96E-08	0.098727	2.20E-07	**0.020136**	4.48E-08
Gould's Petrel	0.106702	4.43E-08	0	0	**0**	0
Hawaiian Petrel	0.190364	8.34E-07	0.156419	4.64E-06	**0.031903**	9.46E-07
Henderson Petrel	0.151388	8.35E-08	0	0	**0**	0
Jamaica Petrel	0.219561	5.52E-06	0.308926	3.08E-05	**0.063008**	6.29E-06
Juan Fernandez Petrel	0.178498	3.00E-07	0.094146	1.67E-06	**0.019202**	3.40E-07
Kerguelen Petrel	0.124948	5.66E-08	0	0	**0**	0
Kermadec Petrel	0.223777	5.47E-07	0.338962	3.06E-06	**0.069134**	6.23E-07
Magenta Petrel	0.21493	2.75E-08	0.294041	1.53E-07	**0.059972**	3.13E-08
Mottled Petrel	0.155456	2.71E-07	0	0	**0**	0

	Murphy's Petrel	0.166846	3.03E-07	0.021778	1.68E-06	**0.004442**	3.43E-07
	Phoenix Petrel	0.13051	3.08E-08	0	0	**0**	0
	White-headed Petrel	0.323449	3.84E-08	0.917005	2.19E-07	**0.18703**	4.47E-08
	White-necked Petrel	0.224259	2.19E-07	0.324201	1.22E-06	**0.066123**	2.50E-07
	Zino's Petrel	0.118975	1.28E-05	0	0	**0**	0
Prions	Antarctic Prion	0.730925	1.46E-07	3.417431	9.03E-07	**0.313432**	3.63E-08
	Blue Petrel	1	7.79E-16	5.105824	4.89E-15	**0.381304**	1.97E-16
	Broad-billed Prion	0.584172	6.42E-07	2.523635	3.87E-06	**0.277503**	1.55E-07
	Fairy Prion	0.54069	5.72E-07	2.256476	3.41E-06	**0.266763**	1.37E-07
	Fulmar Prion	0.548095	1.52E-07	2.302322	9.05E-07	**0.268606**	3.64E-08
	Medium-billed Prion	0.585807	2.85E-07	2.531045	1.72E-06	**0.277801**	6.91E-08
	Thin-billed Prion	0.552472	1.22E-07	2.328895	7.30E-07	**0.269674**	2.94E-08
Procellarine petrels	Bulwer's Petrel	7.01E-09	1.61E-14	0	0	**0**	0
	Fiji Petrel	0.015473	8.89E-08	0	0	**0**	0
	Grey Petrel	0.48884	1.52E-07	2.668124	8.96E-07	**0.283311**	3.60E-08
	Jouanin's Petrel	8.01E-09	2.88E-14	0	0	**0**	0
	Westland Petrel	0.224223	4.92E-08	1.16456	2.75E-07	**0.222869**	1.11E-08
	White-chinned Petrel	0.482049	1.83E-07	2.648351	1.08E-06	**0.282516**	4.34E-08

Shearwaters	Audubon’s Shearwater	0.335111	1.30E-06	1.628319	7.43E-06	**0.241512**	2.99E-07
	Balearic Shearwater	0.498683	4.65E-06	2.602719	2.75E-05	**0.280682**	1.11E-06
	Black-vented Shearwater	0.463721	1.14E-06	2.390109	6.67E-06	**0.272135**	2.68E-07
	Buller’s Shearwater	0.463731	6.60E-07	2.390171	3.88E-06	**0.272138**	1.56E-07
	Christmas Island Shearwater	0.439476	2.64E-07	2.23991	1.54E-06	**0.266097**	6.20E-08
	Cory’s Shearwater	0.29842	9.10E-07	1.464091	5.16E-06	**0.23491**	2.07E-07
	Flesh-footed Shearwater	0.609083	5.23E-07	3.277208	3.17E-06	**0.307796**	1.27E-07
	Fluttering Shearwater	0.421146	4.59E-07	2.134173	2.67E-06	**0.261847**	1.07E-07
	Great Shearwater	0.745696	7.59E-07	4.124458	4.72E-06	**0.341854**	1.90E-07
	Hutton’s Shearwater	0.45124	1.23E-06	2.308762	7.19E-06	**0.268865**	2.89E-07
	Little Shearwater	0.369212	3.75E-07	1.823196	2.16E-06	**0.249346**	8.68E-08
	Manx Shearwater	0.4987	1.03E-06	2.596324	6.12E-06	**0.280425**	2.46E-07
	Short-tailed Shearwater	0.640927	4.98E-07	3.458583	3.03E-06	**0.315087**	1.22E-07
	Sooty Shearwater	0.721503	2.10E-07	3.97433	1.30E-06	**0.335819**	5.22E-08

Townsend's Shearwater	0.461346	3.99E-07	2.365612	2.34E-06	**0.27115**	9.41E-08
Wedge-tailed Shearwater	0.474891	1.29E-07	2.454589	7.60E-07	**0.274727**	3.05E-08
Yelkouan Shearwater	0.493557	0	2.561855	0	**0.279039**	0

Supplementary information

Extended Methodology

This global seabird mortality estimate was achieved by integrating three models, global seabird debris ingestion incidence (Model 1), seabird debris incidence-load conversion (Model 2) and seabird ingested debris load mortality model (Model 3), according to Figure 2. Statistical analyses were performed using R (Version 3.3.3).

Model 1: Global seabird marine debris ingestion incidence data used in this study was sourced by modelled incidence of marine debris by Procellariiformes generated by Wilcox et al. 2015 [1] in SI Appendix, "Supplementary Table 1. Seabirds included in this study, based on published ingestion rates or modelled risk in our study". Available at http://www.pnas.org/content/pnas/suppl/2015/08/27/1502108112.DCSupplemental/pnas.1502108112.sapp.pdf

Model 2: We used a General Additive Model (GAM) to model the relationship between incidence and load of marine debris ingestion in Procellariiform seabirds, using marine debris incidence and load data from 1733 individuals of 51 species (Chapter 2), and weighted results by the number of individuals representing a species in that study. We tested several models including a combination of the incidence, bird group and average species weight, choosing the best model by the lowest AIC. Species average weights were taken as middle weights from a combination of sources [15, 16], and where exact weights were not available, they were estimated based on similarly sized petrels within their genus. The best model included incidence (smooth), species average weight (smooth) and bird group ($P<0.01$, $R2 = 0.902$, deviance explained = 92.2%) (Figure 1). With this incidence-load model, we determined the mean marine debris load ingested by each species.

Model 3: We used the dose-response mortality relationship generated in Chapter 3 to determine both the mean expected mortality for each species as well as this mortality multiplied by the marine debris incidence expected in each 1° latitude by 1° longitude grid [1] across a species foraging range from Bird Life [2].

To estimate global seabird mortality, the debris ingestion incidences from species from Model 1, where data was available, were converted to expected ingested debris loads using Model 2 expected in each 1° latitude by 1° longitude grid [1] across a species foraging range from Bird Life [2]. We then determined the mortality for each 1° latitude by 1° longitude grid [1] for each species using Model 3. For species in a 1°x1° square where the debris load is between 0 and 1, we multiplied the load by 0.2039 (the chance of death from ingesting one item from debris ingestion mortality model, Model 3). For species in a 1°x1° square where the debris load is greater than 1, we used the "Predict" function in R with the debris ingestion mortality model (Model 3) to determine the chance of mortality for each calculated debris load for each 1°x1° square. We then took the mean and standard error for mortalities for all 1°x1° squares and presented these results (Table 1).

References

1. Wilcox C, Van Sebille E, Hardesty BD. Threat of plastic pollution to seabirds is global, pervasive, and increasing. Proc Natl Acad Sci U S A. 2015;112(38):11899-904. doi: 10.1073/pnas.1502108112.
2. Birdlife International and NatureServe. Bird Species Distribution Maps of the World Cambridge, UK: Birdlife International; 2011. Available from: http://datazone.birdlife.org/species/requestdis.
3. Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 2009;364(1526):1985-98. doi: 10.1098/rstb.2008.0205.
4. Lavers JL, Bond AL. Exceptional and rapid accumulation of anthropogenic debris on one of the world's most remote and pristine islands. Proc Natl Acad Sci U S A. 2017;114(23):6052-5. doi: 10.1073/pnas.1619818114.
5. Barnes DK, Walters A, Goncalves L. Macroplastics at sea around Antarctica. Marine Environmental Research. 2010;70(2):250-2. doi: 10.1016/j.marenvres.2010.05.006. PubMed PMID: 20621773.
6. Kukulka T, Proskurowski G, Morét-Ferguson S, Meyer DW, Law KL. The effect of wind mixing on the vertical distribution of buoyant plastic debris. Geophysical Research Letters. 2012;39(7). doi: 10.1029/2012GL051116.
7. Moore CJ. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environmental Research. 2008;108(2):131-9. doi: 10.1016/j.envres.2008.07.025. PubMed PMID: WOS:000260132400002.
8. Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, et al. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLoS ONE. 2014;9(12). doi: 10.1371/journal.pone.0111913.
9. Moret-Ferguson S, Law KL, Proskurowski G, Murphy EK, Peacock EE, Reddy CM. The size, mass, and composition of plastic debris in the western North Atlantic Ocean. Mar Pollut Bull. 2010;60(10):1873-8. doi: 10.1016/j.marpolbul.2010.07.020. PubMed PMID: WOS:000283899500031.
10. Laist DW. Impacts of marine debris: entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. Coe JM, Rogers DB, editors1997. 99-139 p.
11. Derraik JGB. The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull. 2002;44(9):842-52. doi: 10.1016/s0025-326x(02)00220-5. PubMed PMID: WOS:000178361200011.
12. Roman L, Schuyler QA, Hardesty BD, Townsend KA. Anthropogenic debris ingestion by avifauna in eastern Australia. PLoS ONE. 2016;11(8). doi: 10.1371/journal.pone.0158343.
13. Codina-García M, Militão T, Moreno J, González-Solís J. Plastic debris in Mediterranean seabirds. Mar Pollut Bull. 2013;77(1-2):220-6. doi: 10.1016/j.marpolbul.2013.10.002.
14. Acampora H, Lyashevska O, Van Franeker JA, O'Connor I. The use of beached bird surveys for marine plastic litter monitoring in Ireland. Marine Environmental Research. 2016;120:122-9. doi: 10.1016/j.marenvres.2016.08.002.
15. Onley D, Scofield P. Albatrosses, petrels and shearwaters of the world. London: Bloomsbury Publishing; 2007.
16. Marchant S, Higgins PJ. Handbook of Australian, New Zealand and Antarctic Birds. Melbourne: Oxford University Press; 1990a.
17. IUCN. IUCN red list of threatened species: International Union for Conservation of Nature and Natural Resources; 2017 [05/01/2017]. Available from: http://www.iucnredlist.org/.
18. Martin GR. Eye structure and amphibious foraging in albatrosses. Proceedings of the Royal Society B: Biological Sciences. 1998;265(1397):665-71.

19. Tavares DC, de Moura JF, Merico A, Siciliano S. Incidence of marine debris in seabirds feeding at different water depths. Mar Pollut Bull. 2017. doi: 10.1016/j.marpolbul.2017.04.012.
20. Furness RW. Ingestion of plastic particles by seabirds at Gough Island, South Atlantic Ocean. Environmental Pollution Series A: Ecological and Biological. 1985;38(3):261-72.
21. Pierce KE, Harris RJ, Larned LS, Pokras MA. Obstruction and starvation associated with plastic ingestion in a Northern Gannet *Morus bassanus* and a greater shearwater *Puffinus gravis*. Marine Ornithology. 2004;32(2):187-9. PubMed PMID: BIOSIS:PREV200510330842.
22. Connors PG, Smith KG. Oceanic plastic particle pollution - suspected effect on fat deposition in red phalaropes. Mar Pollut Bull. 1982;13(1):18-20. doi: 10.1016/0025-326x(82)90490-8. PubMed PMID: WOS:A1982NB04100005.
23. Colabuono FI, Taniguchi S, Montone RC. Polychlorinated biphenyls and organochlorine pesticides in plastics ingested by seabirds. Mar Pollut Bull. 2010;60(4):630-4. doi: 10.1016/j.marpolbul.2010.01.018. PubMed PMID: 20189196.
24. Ryan PG, Jackson S. The life-span of ingested plastic particles in seabirds and their effect on digestive efficiency. Mar Pollut Bull. 1987;18(5):217-9. doi: 10.1016/0025-326x(87)90461-9. PubMed PMID: WOS:A1987H776600005.
25. Ryan PG. Effects of ingested plastic on seabird feeding: Evidence from chickens. Marine Pollution Bulletin. 1988;19(3):125-8. doi: 10.1016/0025-326X(88)90708-4.
26. Carey MJ. Intergenerational transfer of plastic debris by Short-tailed Shearwaters (*Ardenna tenuirostris*). Emu. 2011;111(3):229-34. doi: 10.1071/mu10085. PubMed PMID: WOS:000294214500008.
27. Bjorndal KA, Bolten AB, Lagueux CJ. Ingestion of marine debris by juvenile sea-turtles in coastal Florida habitats. Mar Pollut Bull. 1994;28(3):154-8. doi: 10.1016/0025-326x(94)90391-3. PubMed PMID: WOS:A1994NC45000009.
28. McCauley SJ, Bjorndal KA. Conservation implications of dietary dilution from debris ingestion: Sublethal effects in post-hatchling loggerhead sea turtles. Conservation Biology. 1999;13(4):925-9. doi: 10.1046/j.1523-1739.1999.98264.x.
29. Naef-Daenzer B, Widmer F, Nuber M. Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. Journal of Animal Ecology. 2001;70(5):730-8. doi: 10.1046/j.0021-8790.2001.00533.x.
30. Mougin JL, Jouanin C, Roux F, Zino F. Fledging weight and juvenile survival of Cory's Shearwaters Calonectris diomedea on Selvagem Grande. Ringing and Migration. 2000;20(2):107-10. doi: 10.1080/03078698.2000.9674231.
31. Yamashita R, Takada H, Fukuwaka MA, Watanuki Y. Physical and chemical effects of ingested plastic debris on short-tailed shearwaters, *Puffinus tenuirostris*, in the North Pacific Ocean. Mar Pollut Bull. 2011;62(12):2845-9. doi: 10.1016/j.marpolbul.2011.10.008. PubMed PMID: 22047741.
32. Giam CS, Chan HS, Neff GS, Atlas EL. Phthalate ester plasticizers: A new class of marine pollutant. Science. 1978;199(4327):419-21.
33. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, et al. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere. 2017;182:781-93. doi: 10.1016/j.chemosphere.2017.05.096.
34. Net S, Sempéré R, Delmont A, Paluselli A, Ouddane B. Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environmental Science and Technology. 2015;49(7):4019-35. doi: 10.1021/es505233b.
35. Oehlmann J, Schulte-Oehlmann U, Kloas W, Jagnytsch O, Lutz I, Kusk KO, et al. A critical analysis of the biological impacts of plasticizers on wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 2009;364(1526):2047-62. doi: 10.1098/rstb.2008.0242.
36. Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. Journal of Steroid Biochemistry and Molecular Biology. 2011;127(3-5):204-15. doi: 10.1016/j.jsbmb.2011.08.007.

37. Kaul AF, Souney PF, Osathanondh R. A review of possible toxicity of di-2-ethylhexylphthalate (DEHP) in plastic intravenous containers: Effects on reproduction. Drug Intelligence and Clinical Pharmacy. 1982;16(9):689-92.
38. Agarwal DK, Lawrence WH, Turner JE, Autian J. Effects of parenteral di-(2-ethylhexyl)phthalate (dehp) on gonadal biochemistry, pathology, and reproductive performance of mice. Journal of Toxicology and Environmental Health. 1989;26(1):39-59. doi: 10.1080/15287398909531232.
39. Bello UM, Madekurozwa MC, Groenewald HB, Aire TA, Arukwe A. The effects on steroidogenesis and histopathology of adult male Japanese quails (Coturnix coturnix japonica) testis following pre-pubertal exposure to di(n-butyl) phthalate (DBP). Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology. 2014;166:24-33. doi: 10.1016/j.cbpc.2014.06.005.
40. Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka MA, Watanuki Y. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Marine Pollution Bulletin. 2013;69(1-2):219-22. doi: 10.1016/j.marpolbul.2012.12.010.
41. Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka MA, Watanuki Y. Facilitated Leaching of Additive-Derived PBDEs from Plastic by Seabirds' Stomach Oil and Accumulation in Tissues. Environmental Science and Technology. 2015;49(19):11799-807. doi: 10.1021/acs.est.5b01376.
42. Marteinson SC, Bird DM, Shutt JL, Letcher RJ, Ritchie IJ, Fernie KJ. Multi-generational effects of polybrominated diphenylethers exposure: Embryonic exposure of male American kestrels (Falco sparverius) to de-71 alters reproductive success and behaviors. Environmental Toxicology and Chemistry. 2010;29(8):1740-7. doi: 10.1002/etc.200.
43. Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, et al. Exposure to polybrominated diphenyl ethers (PBDEs): Changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (Falco sparverius). Toxicological Sciences. 2005;88(2):375-83. doi: 10.1093/toxsci/kfi295.
44. Sullivan KM, Bird DM, Ritchie JI, Shutt JL, Letcher RJ, Fernie KJ. Changes in plasma retinol of American kestrels (Falco sparverius) in response to dietary or in ovo exposure to environmentally relevant concentrations of a penta-brominated diphenyl ether mixture, De-71. Journal of Toxicology and Environmental Health - Part A: Current Issues. 2010;73(23):1645-54. doi: 10.1080/15287394.2010.501720.
45. Engler RE. The complex interaction between marine debris and toxic chemicals in the ocean. Environmental Science & Technology. 2012;46(22):12302-15. doi: 10.1021/es3027105. PubMed PMID: 23088563.
46. Hirai H, Takada H, Ogata Y, Yamashita R, Mizukawa K, Saha M, et al. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Mar Pollut Bull. 2011;62(8):1683-92. doi: 10.1016/j.marpolbul.2011.06.004. PubMed PMID: 21719036.
47. Bakir A, O'Connor IA, Rowland SJ, Hendriks AJ, Thompson RC. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ Pollut. 2016;219:56-65. doi: 10.1016/j.envpol.2016.09.046.
48. Rochman CM, Hoh E, Kurobe T, Teh SJ. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 2013;3. doi: 10.1038/srep03263.
49. Besseling E, Wegner A, Foekema EM, Van Den Heuvel-Greve MJ, Koelmans AA. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environmental Science and Technology. 2013;47(1):593-600.
50. Vegter AC, Barletta M, Beck C, Borrero J, Burton H, Campbell ML, et al. Global research priorities to mitigate plastic pollution impacts on marine wildlife. Endangered Species Research. 2014;25(3):225-47. doi: 10.3354/esr00623.
51. Kühn S, Bravo Rebolledo EL, Van Franeker JA. Deleterious effects of litter on marine life. Marine Anthropogenic Litter: Springer International Publishing; 2015. p. 75-116.

52. Provencher JF, Bond AL, Hedd A, Montevecchi WA, Muzaffar SB, Courchesne SJ, et al. Prevalence of marine debris in marine birds from the North Atlantic. Mar Pollut Bull. 2014;84(1-2):411-7. doi: 10.1016/j.marpolbul.2014.04.044.
53. Ryan PG, de Bruyn PJN, Bester MN. Regional differences in plastic ingestion among Southern Ocean fur seals and albatrosses. Mar Pollut Bull. 2016;104(1-2):207-10. doi: 10.1016/j.marpolbul.2016.01.032.
54. Ryan PG. The incidence and characteristics of plastic particles ingested by seabirds. Marine Environmental Research. 1987;23(3):175-206. doi: 10.1016/0141-1136(87)90028-6. PubMed PMID: WOS:A1987M699900002.
55. van Franeker J. Save the North Sea Fulmar-Litter-EcoQO Manual Part 1: Collection and dissection procedures. Wageningen, Alterra2004.
56. R Core Team. R: A Language and Environment for Statistical Computing Vienna, Austria: R Foundation for Statistical Computing; 2014. Available from: http://www.R-project.org.
57. Reid TA, Tuck GN, Hindell MA, Thalmann S, Phillips RA, Wilcox C. Nonbreeding distribution of flesh-footed shearwaters and the potential for overlap with north Pacific fisheries. Biological Conservation. 2013;166:3-10. doi: 10.1016/j.biocon.2013.06.006.
58. Powell CDL. Foraging movements and the migration trajectory of flesh-footed shearwaters *Puffinus carneipes* from the south coast of Western Australia. Marine Ornithology. 2009;37(2):115-20.
59. Bourne WRP, Imber MJ. Plastic pellets collected by a prion on Gough Island, central South-Atlantic Ocean. Mar Pollut Bull. 1982;13(1):20-1. doi: 10.1016/0025-326x(82)90491-x. PubMed PMID: WOS:A1982NB04100006.
60. Day R, Wehle D, Coleman F, editors. Ingestion of plastic pollutants by marine birds. Proceedings on the workshop of the fate and impact of marine debris; 1985 26-29th November 1984; Honolulu, Hawaii: NOAA Technical Memorandum NMFS.
61. Rapp DC, Youngren SM, Hartzell P, David Hyrenbach K. Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. Mar Pollut Bull. 2017;123(1-2):269-78. doi: 10.1016/j.marpolbul.2017.08.047.
62. Petry MV, Benemann VRF. Ingestion of marine debris by the white-chinned petrel (*Procellaria aequinoctialis*): Is it increasing over time off southern Brazil? Mar Pollut Bull. 2017;117(1-2):131-5. doi: 10.1016/j.marpolbul.2017.01.073.
63. Van Franeker JA, Law KL. Seabirds, gyres and global trends in plastic pollution. Environ Pollut. 2015;203:89-96. doi: 10.1016/j.envpol.2015.02.034.
64. Youngren SM, Rapp DC, Hyrenbach KD. Plastic ingestion by Tristram's Storm-petrel (*Oceanodroma tristrami*) chicks from French frigate shoals, Northwestern Hawaiian Islands. Mar Pollut Bull. 2018;128:369-78. doi: 10.1016/j.marpolbul.2018.01.053.
65. Furtado R, Menezes D, Santos CJ, Catry P. White-faced storm-petrels *Pelagodroma marina* predated by gulls as biological monitors of plastic pollution in the pelagic subtropical Northeast Atlantic. Mar Pollut Bull. 2016;112(1-2):117-22. doi: 10.1016/j.marpolbul.2016.08.031.
66. Copello S, Quintana F, Pérez F. Diet of the southern giant petrel in Patagonia: Fishery-related items and natural prey. Endangered Species Research. 2008;6(1):15-23. doi: 10.3354/esr00118.
67. Croxall JP, Butchart SHM, Lascelles B, Stattersfield AJ, Sullivan B, Symes A, et al. Seabird conservation status, threats and priority actions: A global assessment. Bird Conservation International. 2012;22(1):1-34. doi: 10.1017/S0959270912000020.
68. Baker GB, Gales R, Hamilton S, Wilkinson V. Albatrosses and petrels in Australia: a review of their conservation and management. Emu. 2002;102(1):71-97. doi: 10.1071/mu01036. PubMed PMID: WOS:000175581800007.
69. Kissin YV. Polyethylene, End-Use Properties and their Physical Meaning. Munich: Hanser Publishers; 2013.

70. Rochman CM, Hoh E, Hentschel BT, Kaye S. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environmental Science and Technology. 2013;47(3):1646-54. doi: 10.1021/es303700s.
71. Mahapram S, Poompradub S. Preparation of natural rubber (NR) latex/low density polyethylene (LDPE) blown film and its properties. Polym Test. 2011;30(7):716-25. doi: 10.1016/j.polymertesting.2011.06.006. PubMed PMID: WOS:000295244200004.
72. Bhadeshia H, Honeycombe R. Steels: Microstructure and Properties. 4th Edition ed. Oxford, United Kingdom: Butterworth-Heinemann; 2017.
73. Wilcox C, Puckridge M, Schuyler QA, Townsend K, Hardesty BD. A quantitative analysis linking sea turtle mortality and plastic debris ingestion. Scientific Reports. 2018;8(1). doi: 10.1038/s41598-018-30038-z.
74. Kumar P, Gahlot TK. Clinical evaluation of oesophageal obstruction in bovines. Veterinary Practitioner. 2015;16(2):317-9.
75. Capak D, Brkić A, Harapin I, Matičić D, Radišić B. Treatment of the foreign body induced occlusive ileus in dogs. Veterinarski Arhiv. 2001;71(6):345-59.
76. Miller CL, Bischoff KL, Hoff B. Polyacrylamide gel ingestion leading to fatal intestinal obstruction in two birds in a zoological collection. Journal of Avian Medicine and Surgery. 2009;23(4):286-9. doi: 10.1647/1082-6742-23.4.286.
77. Wilcox C, Mallos NJ, Leonard GH, Rodriguez A, Hardesty BD. Using expert elicitation to estimate the impacts of plastic pollution on marine wildlife. Marine Policy. 2016;65:107-14. doi: 10.1016/j.marpol.2015.10.014.
78. Acampora H, Schuyler QA, Townsend KA, Hardesty BD. Comparing plastic ingestion in juvenile and adult stranded short-tailed shearwaters (Puffinus tenuirostris) in eastern Australia. Mar Pollut Bull. 2014;78(1-2):63-8.
79. Rochman CM, Kurobe T, Flores I, Teh SJ. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of the Total Environment. 2014;493:656-61. doi: 10.1016/j.scitotenv.2014.06.051. PubMed PMID: WOS:000340312000071.
80. ter Halle A, Ladirat L, Martignac M, Mingotaud AF, Boyron O, Perez E. To what extent are microplastics from the open ocean weathered? Environ Pollut. 2017;227:167-74. doi: 10.1016/j.envpol.2017.04.051.
81. Azzarello MY, Van Vleet ES. Marine birds and plastic pollution. Marine Ecology Progress Series. 1987;37:295-303.
82. Fossi MC, Panti C, Baini M, Lavers JL. A Review of Plastic-Associated Pressures: Cetaceans of the Mediterranean Sea and Eastern Australian Shearwaters as Case Studies. Frontiers in Marine Science. 2018;5(173). doi: 10.3389/fmars.2018.00173.
83. EPA. Endocrine Disruptor Screening Program Test Guidelines. OCSPP 890.2100: Avian Two-generation toxicity test in the Japanese Quail. In: Agency USEP, editor. 2015.
84. Parsley LM, Wapstra E, Jones SM. Yolk contributes steroid to the multidimensional endocrine environment of embryos of Niveoscincus metallicus, a viviparous skink with a moderately complex placenta. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. 2014;171:51-6. doi: 10.1016/j.cbpa.2014.02.013.
85. Jovanović B, Gökdağ K, Güven O, Emre Y, Whitley EM, Kideys AE. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar Pollut Bull. 2018;130:123-31. doi: 10.1016/j.marpolbul.2018.03.016.
86. Parsley LM, Wapstra E, Jones SM. *Atrazine Disrupts Gonadal development in a live-bearing lizard*. Endocrine Disruptors. 2015;3(1):e100607-1-10. doi: 10.1080/23273747.2015.1006071.
87. Hodges RD, Hanley HG. Malformations of the epithelium of the ductus epididymidis. Journal of reproduction and fertility. 1969;20(1):45-50. doi: 10.1530/jrf.0.0200045.

88. Dunn TB, Green AW. Cysts of the epididymis, cancer of the cervix, granular cell myoblastoma, and other lesions after estrogen injection in newborn mice. Journal of the National Cancer Institute. 1963;31(2):425-55. doi: 10.1093/jnci/31.2.425.
89. Granados H, Dam H. Testicular hypoplasia and epididymal cysts in the Syrian hamster [10]. Nature. 1948;162(4112):297. doi: 10.1038/162297a0.
90. Hayes TB, Anderson LL, Beasley VR, De Solla SR, Iguchi T, Ingraham H, et al. Demasculinization and feminization of male gonads by atrazine: Consistent effects across vertebrate classes. J Steroid Biochem Mol Biol. 2011;127(1-2):64-73.
91. Ciacciariello M, Gous RM. A comparison of the effects of feeding treatments and lighting on age at first egg and subsequent laying performance and carcase composition of broiler breeder hens. British Poultry Science. 2005;46(2):246-54. doi: 10.1080/00071660500066233.
92. Madnurkar AD, Shinde AS, Chouhan L, Singh V, Mohan J, Moudgal RP. Effect of dietary phytoestrogens, feed restriction, and their interaction on reproductive status of broiler pullets. Veterinary World. 2014;7(12):1041-6. doi: 10.14202/vetworld.2014.1041-1046.
93. Lavers JL, Bond AL, Hutton I. Plastic ingestion by flesh-footed shearwaters (Puffinus carneipes): Implications for fledgling body condition and the accumulation of plastic-derived chemicals. Environ Pollut. 2014;187:124-9. doi: 10.1016/j.envpol.2013.12.020.
94. Lavers JL, Hutton I, Bond AL. Ingestion of marine debris by Wedge-tailed Shearwaters (Ardenna pacifica) on Lord Howe Island, Australia during 2005–2018. Mar Pollut Bull. 2018;133:616-21. doi: 10.1016/j.marpolbul.2018.06.023.
95. Herzke D, Anker-Nilssen T, Nøst TH, Götsch A, Christensen-Dalsgaard S, Langset M, et al. Negligible Impact of Ingested Microplastics on Tissue Concentrations of Persistent Organic Pollutants in Northern Fulmars off Coastal Norway. Environmental Science and Technology. 2016;50(4):1924-33. doi: 10.1021/acs.est.5b04663.
96. Provencher JF, Avery-Gomm S, Liboiron M, Braune BM, Macaulay JB, Mallory ML, et al. Are ingested plastics a vector of PCB contamination in northern fulmars from coastal Newfoundland and Labrador? Environmental Research. 2018;167:184-90. doi: 10.1016/j.envres.2018.07.025.
97. Ainley DG, Dugger KM, Mesa ML, Ballard G, Barton KJ, Jennings S, et al. Post-fledging survival of Adélie penguins at multiple colonies: Chicks raised on fish do well. Marine Ecology Progress Series. 2018;601:239-51. doi: 10.3354/meps12687.
98. Arizaga J, Herrero A, Aldalur A, Cuadrado JF, Oro D. Effect of Pre-Fledging Body Condition on Juvenile Survival in Yellowlegged Gulls Larus michahellis. Acta Ornithologica. 2015;50(2):139-47. doi: 10.3161/00016454AO2015.50.2.002.
99. Janinhoff N, Verdaat H, van Franeker JA. Grey phalarope foraging in plastic soup. Sula. 2010;23(1):41-5. PubMed PMID: ZOOREC:ZOOR14610073526.
100. Newton KM, Croll DA, Nevins HM, Benson SR, Harvey JT, Tershy BR. At-sea mortality of seabirds based on beachcast and offshore surveys. Marine Ecology Progress Series. 2009;392:295-305. doi: 10.3354/meps08152.
101. Tavares DC, De Moura JF, Siciliano S. Environmental predictors of seabird wrecks in a tropical coastal area. PLoS ONE. 2016;11(12). doi: 10.1371/journal.pone.0168717.
102. Ryan PG. The effects of ingested plastic on seabirds- correlatios between plastic load and body condition. Environ Pollut. 1987;46(2):119-25. doi: 10.1016/0269-7491(87)90197-7. PubMed PMID: WOS:A1987J374900003.
103. van Franeker JA, Blaize C, Danielsen J, Fairclough K, Gollan J, Guse N, et al. Monitoring plastic ingestion by the northern fulmar *Fulmarus glacialis* in the North Sea. Environ Pollut. 2011;159(10):2609-15. doi: 10.1016/j.envpol.2011.06.008. PubMed PMID: WOS:000295493100051.
104. Roman L, Wilcox C, Hardesty BD, Hindell M. Are young seabirds at greater risk of harm by plastic pollution? Submitted. 2018.
105. Sæther BE, Bakke Ø. Avian life history variation and contribution of demographic traits to the population growth rate. Ecology. 2000;81(3):642-53.

106. Maness TJ, Anderson DJ. Predictors of juvenile survival in birds. Ornithological Monographs 2013. p. 1-55.